

■ R E V I E W

Angiotensin receptor blockers and angiogenesis: clinical and experimental evidence

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A B S T R A C T

Angiotensin II type I receptor antagonists [ARBs (angiotensin receptor blockers)] are indicated for BP (blood pressure)-lowering, renal protection and cardioprotection in patients unable to tolerate ACEIs (angiotensin-converting enzyme inhibitors). A recent meta-analysis revealed an association between ARBs and tumour development, possibly due to enhancement of angiogenesis. However, published evidence is conflicting on the effects of ARBs on angiogenesis or the expansion of the existing vascular network. ARBs have been shown to exert primarily anti-angiogenic effects in basic science studies of cancer, retinopathy, peripheral artery disease and some models of cardiovascular disease. In animal and cellular models of myocardial infarction and stroke, however, ARB administration has been associated with robust increases in vascular density and improved recovery. The aim of the present review is to examine the angiogenic effects of ARBs in animal and cellular models of relevant disease states, including proposed molecular mechanisms of action of ARBs and the clinical consequences of ARB use.

INTRODUCTION

ARBs (angiotensin receptor blockers)

The RAS (renin–angiotensin system) plays a central role in the control of BP (blood pressure), blood volume and electrolyte balance. When blood volume or BP is low, the kidneys secrete renin, which activates the cleavage of angiotensinogen to produce AngI (angiotensin I). AngI is in turn converted into AngII (angiotensin II) by ACE (angiotensin-converting enzyme). AngII then initiates arteriolar vasoconstriction, restoring BP. Therapeutically, the RAS can be manipulated at three points to effect a reduction in BP: aliskiren inhibition of renin production, ACE inhibition to block AngII formation, and antagonism of the AT₁R (AngII type 1

receptor) by ARBs [1]. ARBs include losartan (Cozaar[®]), valsartan (Diovan[®]), candesartan (Atacand[®]), irbesartan (Avapro[®]), telmisartan (Micardis[®]), eprosartan (Teveten[®]) and olmesartan (Benicar[®]). Structurally, ARBs resemble the prototypical losartan, containing biphenyl and tetrazole moieties, with the exception of eprosartan [2]. In terms of receptor affinity, candesartan and olmesartan are stronger antagonists than irbesartan, which has an affinity approximately equal to that of eprosartan. Next in line are telmisartan and valsartan, followed by losartan [3]. ARBs were originally developed as antihypertensive medications, but recent studies have demonstrated that ARBs can have an impact upon a number of other physiological processes, including the generation of new vasculature from existing blood vessels,

Key words: angiogenesis, angiotensin II type 1 receptor (AT₁R), angiotensin receptor blocker (ARB), renin–angiotensin system (RAS), vascular endothelial growth factor (VEGF).

Abbreviations: ACE, angiotensin-converting enzyme; ACEI, ACE inhibitor; AngI, angiotensin I; AngII, angiotensin II; ARB, angiotensin receptor blocker; AT₁R, AngII type 1 receptor; AT₂R, AngII type 2 receptor; BP, blood pressure; CHARM, Candesartan in Heart failure Assessment of Reduction in Mortality and Morbidity; CI, confidence interval; CSF, cerebrospinal fluid; DIRECT, Diabetic Retinopathy Candesartan Trials; EGF, epidermal growth factor; EGFR, EGF receptor; ETDRS, Early Treatment Diabetic Retinopathy Study; FDA, Food and Drug Administration; FGF, fibroblast growth factor; HIF, hypoxia-inducible transcription factor; HR, hazard ratio; Hsp, heat-shock protein; ICAM-1, intercellular cell adhesion molecule-1; IDNT, Irbesartan Diabetic

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a process termed angiogenesis [4]. As a reparative process, angiogenesis could be exploited to treat conditions associated with ischaemia such as heart disease and stroke. However, increased angiogenesis is most often associated with pathological conditions, including cancer, ocular disease, obesity, arthritis, psoriasis and inflammatory bowel disease [5]. Studies concerning the exact role of ARBs in angiogenesis have been contradictory: some studies have revealed a profound anti-angiogenic effect of AT₁R blockade, whereas others have demonstrated increased angiogenesis as a result of ARB administration. In addition, a recent meta-analysis of ARB use in clinical trials implies that an observed increase in lung cancer risk could involve angiogenic processes mediated by ARBs [6], whereas basic science evidence clearly demonstrates an anti-angiogenic action of ARBs in cancer models [7]. The scope of the present review is therefore to discuss the pro- and anti-angiogenic effects of ARBs in animal and cellular models of relevant disease states. The evidence is summarized in Table 1.

Angiogenesis and angiotensin

Angiogenesis is a highly regulated process dependent on the actions of numerous proteases, growth factors and signalling cascades. In order for angiogenesis to proceed, the original vascular structure must be broken down and re-formed. This process involves all components of the vasculature, including endothelial cells, pericytes, fibroblasts, smooth muscle cells and the extracellular matrix. Vessels must first be destabilized and normally quiescent endothelial cells transformed into a proliferative phenotype. Transformation is followed by degradation of the endothelial cell basement membrane and dissolution of the surrounding extracellular matrix through the actions of tissue proteinases, allowing for endothelial cell proliferation and migration [8]. Migration is directed towards a stimulus, for example the growth factors VEGF (vascular endothelial growth factor) or EGF (epidermal growth factor) [9]. Following migration, endothelial cells establish stable cell–cell contacts via the expression of cell-surface proteins, including cadherins and ephrins, and form tubes through which blood can flow. Finally, mesenchymal cells migrate along newly formed vessels and differentiate into mature pericytes

Table 1 ARBs have both pro- and anti-angiogenic actions in basic science studies of disease models

Effects of ARBs	Reference(s)
Anti-angiogenic	
Cancer	
Lung	[26,42,120]
Brain	[32]
Skin	[36]
Ovarian	[23]
Pancreatic	[31]
Gastric	[29,30]
Colorectal	[121]
Bladder	[21,25]
Prostate	[28,49]
Retinopathy	
Premature	[68–71]
Injury	[77]
Diabetes	[74,75]
Hypertension	[76]
PAD	
Hypertension	[11,88]
Ischaemia	[89–91,122]
Heart disease	
Neonatal heart disease	[93]
Congestive heart failure	[92]
Diabetes	[94]
Pro-angiogenic	
MI	[98–100]
Brain	[13–15,108,114]
Alginate implant model	[43]

[10]. Endothelial cells once again become quiescent, cell contacts are strengthened and the stability of the new vessel is established.

The RAS appears to be involved in both rarefaction (structural degeneration) and expansion of the vascular network. Initial studies revealed that, when rats were administered a high-salt diet along with infusion of AngII, vascular density in the cremaster muscle was increased; this increase was potentiated by co-infusion of an AT₂R (AngII type 2 receptor) antagonist, but decreased when

Abbreviations cont. Nephropathy Trial; IDR, incidence density ratio; IL, interleukin; LIFE, Losartan Intervention For Endpoint reduction in hypertension; MAPK, mitogen-activated protein kinase; MCAO, middle cerebral artery occlusion; MCP-1, monocyte chemoattractant protein-1; MI, myocardial infarction; MOSES, MOrbidity and mortality after Stroke, Eprosartan compared with nitrendipine for Secondary prevention; NF- κ B, nuclear factor κ B; OIR, oxygen-induced retinopathy; ONTARGET, ONGoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial; PAD, peripheral artery disease; PI3K, phosphoinositide 3-kinase; PPAR γ , peroxisome-proliferator-activated receptor γ ; PRoFESS, Prevention Regimen For Effectively avoiding Second Strokes; PSA, prostate-specific antigen; RAS, renin-angiotensin system; RENAAL, Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan; ROP, retinopathy of prematurity; RR, relative risk; TRANSCEND, Telmisartan Randomized Assessment Study in ACE iNtolerant subjects with cardiovascular Disease; VALUE, Valsartan Antihypertensive Long-term Use Evaluation; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

the AT₁R antagonist losartan was administered [11]. Although ARBs lower BP, which in turn can induce vascular remodelling resulting in more blood vessels, the dynamic vascular processes of degeneration and regeneration during angiogenesis have been more tightly correlated to levels of circulating AngII than BP [4].

Circulating AngII can either bind to the AT₁R or AT₂R on endothelial cells. AngII–AT₁R interactions are the most widely studied, and stimulation of AT₁R by AngII has been shown to initiate a series of signalling cascades resulting in the expression of growth factors such as VEGF, FGF (fibroblast growth factor), IGF (insulin-like growth factor) and TGF β (transforming growth factor β) [4]. AT₁R activation by AngII will also initiate signalling cascades leading to increased expression of the early response genes *c-fos*, *c-jun* and *c-myc*, which are involved in extracellular matrix remodelling and cellular proliferation [12]. The actions of AngII on the AT₂R are less well understood, but studies have shown that AT₂R stimulation leads to cellular differentiation and apoptosis [4]. The long-held belief that blocking the AngII interaction with AT₁R will inevitably inhibit angiogenesis has recently come under question with the publication of several studies indicating that, in certain circumstances, ARB treatment is actually associated with increased blood vessel formation [13–15].

ANTI-ANGIOGENIC ACTIONS OF ARBs

Cancer: experimental evidence

Just as normal tissues in the body require vascular support for nutrition and survival, so do cancerous tumours. Endothelial cells get transformed when stimulated by tumour-derived factors and proliferate at a rate approx. 40 times higher than normal endothelial cells [16]. These ‘hyper-activated’ endothelial cells are essential for the initiation of tumour perfusion and micrometastasis [17]. Hence inhibition of tumour angiogenesis, a process known as anti-angiogenic therapy, has become a major focus of cancer treatment. Tumour cells can commandeer the existing capillary network and induce self-neovascularization through mechanisms similar to physiological angiogenesis. As an avascular tumour grows, cells at the innermost core of the tumour lose vascular contact with nearby blood vessels and experience hypoxia. This hypoxic episode leads the cells to release angiogenic factors such as VEGF, FGF and IL (interleukin)-8, coaxing vascular endothelial cells to divide, migrate and extend new vessels into the tumour mass [18]. Just as in physiological angiogenesis, tumour-mediated angiogenesis relies heavily on VEGF, which is up-regulated in most human cancers [19].

AT₁R blockers have been implicated in tumour growth inhibition by inducing apoptosis [20], as well as inhibiting cellular proliferation and vascular invasion. However, the

main effect of ARBs on cancer appears to be mediated through inhibition of the release of pro-angiogenic factors from tumour cells *in vitro*, observed *in vivo* as a lower density of blood vessels and reduced tumour volume [21,22]. Tumour size has been shown to be sensitive to ARB treatment in a number of animal models of cancer, including ovarian [23], breast [24], bladder [25], lung [26,27], prostate [28], gastric [29,30], pancreatic [31], sarcoma [27] and glioma [32]. In nearly all animal experiments, ARB administration was associated with a reduced expression of VEGF in tumours regardless of tumour type. Candesartan administration decreased not only VEGF, but also the angiogenic factors IL-8 [25], HIF (hypoxia-inducible transcription factor)-2 α [33] and EGF [26] in various cancer cell lines. In rat glioma cells, losartan was shown to decrease tumour production of PDGF (platelet-derived growth factor) and bFGF (basic FGF) in addition to VEGF [34]. Treatment of hormone-refractory prostate cancer cells with ARBs significantly inhibited VEGF production, which is mediated via inhibition of the HIF-1 α and Ets-1 transcription factors [35]. One study of murine melanoma found that losartan treatment decreased tumour growth, but this effect was not associated with changes in VEGF expression. Instead, losartan treatment was associated with a decrease in the expression of the VEGFR1 (VEGF receptor 1 or Flt-1) and reduced mRNA levels of endothelial receptor CD31 [or PECAM-1 (platelet/endothelial cell adhesion molecule-1)], together resulting in reduced vasculature in the tumour tissue [36]. Treatment with candesartan (TCV-116) also had a tendency to decrease protein expression of VEGFR1 and EGFR (EGF receptor) in lung carcinoma cells [26]. Lung tumour xenografts in AT_{1a}^{-/-} mice (mice lacking AT_{1a}Rs) had reduced mRNA levels of VEGF, VEGFR1, VEGFR2, EGF, EGFR and angiopoietin-1 [26]. In the same study, the authors showed that treatment with candesartan in the AT_{1a}^{-/-} mice resulted in a further decrease in angiogenesis, VEGF expression and tumour growth. Functions mediated by VEGFR1 are poorly understood, but stimulation by VEGF-A (often referred to as simply VEGF) or VEGF-B (which is prominent during embryonic development) could induce recruitment of endothelial cell progenitors from bone marrow [37]. VEGFR1 is up-regulated in certain cancers, and is associated with migration and invasion in pancreatic cancer cells [38]. ARBs therefore have the potential to affect several aspects of angiogenesis in cancer progression: reducing the induction of neovascularization via inhibition of pro-angiogenic factor release and decreasing the responsiveness of the vasculature to pro-angiogenic factors by regulating VEGFR expression.

In addition to angiogenic progression, ARBs have the potential to affect other cancer-related processes such as growth arrest and apoptosis. Apoptosis was increased in a leukaemia cell line following exposure

to losartan [39]. Telmisartan inhibited proliferation in prostate cancer cells, but this effect was not observed with candesartan, valsartan, irbesartan or losartan [40]. However, a recent study indicates that losartan may increase apoptosis in human prostate cancer cells [41]. Several studies have demonstrated that candesartan did not affect cell viability [21,25,26] or proliferation [28] *in vitro*. ARBs have also been observed to have an impact upon the invasive potential of tumour cells. In human ovarian cancer tissue, treatment with candesartan reduced tumour vascular density and inhibited the peritoneal dissemination of transplanted ovarian cancer cells [23]. Additionally, candesartan has been shown to prevent pulmonary metastasis of renal cancer cells [42] and lung metastasis of intravenously injected lung carcinoma cells [27].

The molecular mechanisms by which AT₁R antagonists inhibit cancer cell proliferation and secretion of pro-angiogenic growth factors and cytokines are still not very well characterized. Researchers initially believed that the ability of ARBs to regulate BP and vascular tone could be the major reason for observed effects on the tumour vasculature. In a mouse model of gastric cancer, losartan and valsartan inhibited VEGF expression, lymphatic microvessel density and tumour growth to the same degree as the ACEIs (ACE inhibitors) perindopril and captopril [29]. The anti-angiogenic effects of candesartan on tumour growth of sarcoma and fibrosarcoma cells was also observed following treatment with the ACEI lisinopril [27]. Additionally, the anti-cancer effects of ARBs could involve increased binding of AngII to other receptors. Candesartan decreased VEGF production in CV-4 prostate cancer cells, but only when the cells were also stimulated with AngII [28]. However, only selected (and not all, see [43,44]) ARBs inhibit tumour angiogenesis and cancer progression, which challenged this initial hypothesis and revealed that ARBs could function as more than merely BP-lowering agents. AngII has been shown to induce the secretion of pro-angiogenic and pro-inflammatory cytokines IL-6, IL-1 α , IL-8 and MCP-1 (monocyte chemoattractant protein-1) by prostate cancer cells [45], suggesting that ARBs might block the secretion of these cytokines to inhibit tumour angiogenesis. AngII has been shown to stimulate angiogenesis via activation of PI3K (phosphoinositide 3-kinase)/Akt signalling and to inhibit apoptosis via a reduction in caspase 3 activity [46]. Hence AT₁R blockers may also have direct effects on tumour endothelial cells [47]. ARBs are known to suppress growth-factor-mediated cell signalling in endothelial cells, including EGF-, VEGF- and angiopoietin-1-associated signalling, leading to angiogenesis [48]. CV11974, an active metabolite of TCV116 (candesartan), has been shown to inhibit the tyrosine kinase activity of EGFR in prostate cancer cells, thus inhibiting MAPK (mitogen-activated protein kinase) signalling and STAT3 (signal transducer

and activator of transcription 3) phosphorylation [49]. Oral administration of CV11974 resulted in decreased prostate cancer cell proliferation, reduced tumour growth and decreased vascular density in a tumour xenograft model [49].

In addition, a study on the effect of ARBs in prostate cancer has demonstrated that telmisartan exhibited a similar function as pioglitazone, a specific ligand of PPAR γ (peroxisome-proliferator-activated receptor γ) [50]. In prostate cancer (PC3) cells, treatment with telmisartan, but not other ARBs, inhibited proliferation via activation of PPAR γ [40]. However, a recent study has demonstrated that telmisartan exposure increased proliferation of endothelial progenitor cells through the PI3K/Akt pathway [51]. The anti-proliferative mechanism was supported by another study showing that telmisartan-induced prostate cancer cell apoptosis was mediated by increased MAPK and PPAR γ activity, which, in turn, resulted in the binding of PPAR γ to a PPRE (PPAR-responsive element) at the PSA (prostate-specific antigen) promoter and reduced expression of PSA [52]. ARBs have also been shown to inhibit VEGF secretion by prostate cancer cells via inhibition of HIF-1 and Ets-1 [35].

Basic science studies have thus far demonstrated several mechanisms of action of ARBs on angiogenic processes in cancer models and cancer cells. Tumour size has been shown to be sensitive to ARB exposure, as has tumour microvessel density. ARBs have also been shown to inhibit the expression of VEGF and its receptor in cancer cells, inhibiting cell growth, proliferation and metastasis. More in-depth studies on the molecular mechanisms regulating ARB-mediated inhibition of tumour progression are necessary, but present evidence supports the influence of ARBs on a number of cellular signalling cascades. In spite of a number of studies demonstrating the anti-angiogenic effects of ARBs in cancer models, a few studies have shown that ARBs can have no effect or can even increase vascular density in cancer models. A recent study suggested that candesartan treatment of mice implanted with LL/2 carcinoma cells increased tumour size after 21 days, but resulted in no significant difference in tumour volume after 28 days [44]. The effects of candesartan on angiogenesis were not explored in that study. Losartan administration in the alginate implant model of tumour angiogenesis was associated with increased vascular density, and this increase was dependent on intact AT₂R function [43]. The alginate implant angiogenesis model is composed of malignant cells suspended within a bead matrix along with a continuously released cocktail of growth factors known to stimulate angiogenesis, and therefore offers a unique perspective on ARB action regardless of fluctuating growth factor levels. The study by Walther et al. [43] reveals that the effects of ARBs on tumour angiogenesis are still being unravelled.

Cancer: clinical observations

Despite approx. 15 years of clinical use, the safety of ARBs has recently come under scrutiny. Sipahi et al. [6] conducted a recent meta-analysis proposing a modest increased risk of cancer in patients receiving ARBs. The first investigation to propose this was the CHARM (Candesartan in Heart failure Assessment of Reduction in Mortality and Morbidity) programme study in 2003 [53]. In that study, there were more non-cardiovascular deaths reported in the candesartan group than in the placebo group (195 compared with 176; $P = 0.45$), which was due to cancer deaths (86 compared with 59; $P = 0.038$) [53]. Furthermore, updated data from the LIFE (Losartan Intervention For Endpoint reduction in hypertension) study revealed an increased risk of lung cancer {0.6% for losartan compared with 0.3% for atenolol; RR (relative risk) = 2.41 [95% CI (confidence interval), 1.23–4.71]; $P = 0.01$ } [6]. ONTARGET (ONgoing Telmisartan Alone and in combination with Ramipril Global Endpoint Trial) [54] investigated the effects of telmisartan (80 mg/day) or ramipril (5 mg/day) alone or in combination in patients with vascular events or high-risk diabetes. A secondary outcome of that study included the incidence of new cancers. Patients who had received both telmisartan and ramipril had a higher occurrence of new cancers than those who received ramipril alone [9.7 compared with 8.6%; HR (hazard ratio), 1.14 (95% CI, 1.03–1.24); $P = 0.011$] [6,54,55]. In this trial, the concomitant use of ramipril was excluded, and patients who received telmisartan still had a significant risk of increased cancers [8.2 compared with 7.6%; RR, 1.08 (95% CI, 1–1.16); $P = 0.041$] [6]. This was consistent with data published from the TRANSCEND (Telmisartan Randomized Assessment Study in ACE iNtolerant subjects with cardiovascular Disease) trial [56] (telmisartan compared with placebo); patients had an increased risk of all cancers when receiving ARB therapy compared with placebo [236 (8.0%) compared with 204 (6.9%) respectively; RR, 1.16 (95% CI, 0.97–1.39); $P = 0.099$] [6]. In contrast, patients in the PRoFESS (Prevention Regimen For Effectively avoiding Second Strokes) trial [57], who were receiving telmisartan (80 mg/day) or placebo for hypertension reduction following a recent (<90 days) ischaemic stroke, did not show an increase in new cancers [326 (3.3%) compared with 340 (3.4%); RR, 0.96 (95% CI, 0.83–1.12); $P = 0.610$] [6]. Sipahi et al. [6] have commented on specific trials that were not included in their meta-analysis, including the VALUE (Valsartan Antihypertensive Long-term Use Evaluation) trial [58], RENAAL (Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan) trial [59] and IDNT (Irbesartan Diabetic Nephropathy Trial) [60]. When originally contacted, the VALUE investigators claimed to not have collected cancer incidence data. In the RENAAL trial [59], losartan was compared with placebo

in 1513 patients with diabetic nephropathy. Investigators only collected cancer data that led patients to withdrawal from study (four patients on losartan and three patients on placebo). Cancer data was not reported in IDNT [60].

A meta-analysis of all available trials (LIFE, CHARM, TRANSCEND, ONTARGET and PRoFESS) indicates an increased risk [361 (0.9%) compared with 195 (0.7%); RR, 1.25 (95% CI, (1.05–1.49); $P = 0.01$] in the development of lung cancers in patients who have received ARBs. A proposed mechanism for this is that AT₁R blockade through the use of an ARB results in AT₂R stimulation, which thereby increases the production of VEGF [44]. Clinically, VEGF down-regulation has been targeted in solid malignancies (colon, non-small-cell non-squamous cell lung, breast, glioblastoma and renal cell carcinoma) with bevacizumab (Avastin®) [61–65]. Bevacizumab is a humanized monoclonal antibody directed against all isoforms of VEGF-A. The addition of bevacizumab to carboplatin and paclitaxel resulted in an increased survival of 3 months (12.3 compared with 10.3 months; $P = 0.003$) and progression-free survival of 1.7 months (6.2 compared with 4.5 months; $P < 0.001$) in non-small-cell lung cancer patients [61]. In breast cancer, bevacizumab received a controversial FDA (Federal Drug Administration) accelerated approval following the completion of the E2100 trial [63]. In that study, the addition of bevacizumab improved progression-free survival (11.8 compared with 5.9 months; HR for progression, 0.60; $P < 0.001$), but did not significantly improve overall survival (26.7 compared with 25.2 months; HR, 0.88; $P = 0.16$) [63]. Recently presented data from the AVADO and RIBBON-1 trials to the FDA have shown that bevacizumab had less progression-free survival than that published in the E2100 trial, and that, in both studies, the drug did not have a survival benefit [66]. Thus the future of bevacizumab in the treatment of breast cancer is unclear. This is of particular interest given the meta-analysis breast cancer data from Sipahi et al. [6]. Unlike the increased risk of lung cancers found in ARB-based clinical trials, breast cancer did not have an increased risk [154 (1.2%) compared with 119 (1.1%); RR, 1.04 (95% CI, 0.82–1.32); $P = 0.74$] [6]. This may broadly suggest that the involvement of VEGF in tumorigenesis is less in breast cancer when compared with lung cancer. Ongoing clinical trials evaluating the use of bevacizumab earlier in therapy and in differing chemotherapeutic combinations may provide insight into improved strategies to reduce tumour angiogenesis.

Retinopathy: experimental evidence

Several ocular disorders share a common pathological feature of excess vascularization, including age-related macular degeneration, diabetic retinopathy, retinopathy

of prematurity and ocular tumours [67]. These diseases share a common pattern of ischaemic retinopathy, whereby vascular cell death and functional capillary loss triggers the release of angiogenic factors and stimulates neovascularization. One model of proliferative vascular retinopathy, termed OIR (oxygen-induced retinopathy), models a disorder observed in preterm human infants and involves exposure of neonatal rodents to a period of excess oxygen followed by normoxia. The cycle of hyperoxia and hypoxia in OIR results in increased blood vessel growth on the inner surface of the retina. Administration of the AT₁R antagonist losartan [68,69] or valsartan [70] prevented excess neovascularization in the OIR model. Telmisartan administration in the OIR model was shown to have a dual effect on retinal vascularization. Following ischaemia, telmisartan treatment was associated with re-growth of blood vessels into the ischaemic area without the excess pathological revascularization characteristic of retinopathy [71]. The restoration of physiological revascularization without pathological angiogenesis was attributed to the fact that telmisartan treatment was also associated with decreased retinal expression of ICAM-1 (intercellular cell adhesion molecule-1). ICAM-1 is a pro-inflammatory protein up-regulated in retinal diseases [72] and is thought to contribute primarily to pathological retinal neovascularization. Telmisartan may therefore exert distinct effects on angiogenic processes depending on other cellular cues. In addition to inhibiting pathological angiogenesis, olmesartan was shown to attenuate vascular permeability in the OIR model [73]. Other models of retinopathy have also demonstrated an anti-angiogenic effect of ARBs, including candesartan [74] and valsartan in a model of diabetic retinopathy [75] and losartan in hypertension-induced vasculopathy [76] (see Table 1). Even without ischaemic injury, losartan treatment improved vascular morphology and decreased retinal endothelial apoptosis in spontaneously hypertensive rats [76]. Additionally, losartan given in the drinking water was shown to inhibit the development of laser-induced neovascularization of the choroid layer in the eye of mice [77].

The mechanism of action of ARBs on neovascularization in ocular disorders has centred primarily on VEGF. Candesartan administration decreased VEGF levels in the eyes of diabetic rats [74,78,79]. Both telmisartan and valsartan decreased diabetes-induced expression of VEGF and ICAM-1 [80]. Lowered ICAM-1 expression in retinal endothelial cells led to reduced vascular adhesion of leucocytes, reducing inflammation in the diabetic retina [71]. Conversely, losartan was shown to have no effect on *VEGF* mRNA in proliferating blood vessels in a rat model of ROP (retinopathy of prematurity) [68]. ARBs have also been shown to have an impact upon endothelial cell number, as losartan administration in a mouse model of ROP significantly

reduced hypoxia-induced endothelial cell proliferation [69]. Valsartan also reduced endothelial cell proliferation in a diabetic rat model [75]. Cell-cell contact could also be a target of ARBs, as VEGF-induced permeability of ocular vessels was prevented in mice given candesartan [81]. In addition to effects on retinal endothelia, the ARB valsartan was shown to decrease reactivity of Müller cells, specialized glial cells in the retina that secrete VEGF and regulate retinal vascularization [70].

Although the mechanisms of action of ARBs in retinopathy most probably include effects on VEGF, other molecular mechanisms of ARB treatment are beginning to come to light. A recent study identified a number of retinal proteins affected by candesartan treatment in a diabetic mouse model; the majority of proteins normalized by candesartan included those involved in metabolism, energy production and apoptosis [82]. Candesartan was shown to limit accumulation of the oxidative stress-associated AGE (advanced glycation end-product) pentosidine [78] and to decrease the expression of the p22^{phox} subunit of NADPH oxidase, a cellular enzyme responsible for production of ROS (reactive oxygen species) [79].

Retinopathy: clinical observations

In non-diabetic patients and patients with quiescent retinopathy, VEGF levels in the eye are low. However, in patients with proliferating retinopathy, VEGF expression is elevated in ocular fluid [83]. Patients with proliferative retinopathy also demonstrated a strong correlation between elevated VEGF and AngII in ocular fluid, suggesting that inhibition of AngII and VEGF could improve retinopathy [84]. The impact of candesartan (16–32 mg/day) on prevention and treatment of retinopathy in diabetic patients was assessed in the randomized double-blind parallel-design placebo-controlled DIRECT (DIabetic REtinopathy Candesartan Trials) programme [85]. DIRECT-Prevent 1 assessed the progression of retinopathy, as measured by a two or three step increase in the ETDRS (Early Treatment Diabetic Retinopathy Study) scale in Type 1 diabetic patients receiving candesartan ($n = 951$) or placebo ($n = 954$) over 6 years. Results indicated that when retinopathy was already established in patients, candesartan administration did not affect the progression of the disease (HR, 0.80–1.31; $P = 0.85$). In the same study, the authors investigated the incidence of retinopathy in Type 1 diabetic patients (DIRECT-Prevent 1) and found that retinopathy developed in 31% of patients on placebo (217 out of 710), but only 25% of patients on candesartan (178 out of 711). This difference was found to be significant (HR, 0.48–0.87; $P = 0.0034$) even after adjustment for baseline characteristics (HR, 0.53–0.95; $P = 0.046$) [85]. A third arm of DIRECT (DIRECT-Protect 2) investigated the progression of retinopathy in patients with Type 2 diabetes. Retinopathy

in patients maintained on candesartan ($n = 951$) or placebo ($n = 954$) was quantified according to the ETDRS scale. Patients on candesartan exhibited a non-significant reduction in the risk of retinopathy progression compared with patients on placebo [HR, 0.87 (95 % CI, 0.70–1.08); $P = 0.2$]. However, by the end of the trial, candesartan use was associated with less severe retinopathy compared with placebo [OR (odds ratio), 1.17 (95 % CI, 1.05–1.30); $P = 0.003$] [86].

PAD (peripheral artery disease): experimental data

PAD arising from atherosclerosis, inflammatory processes, hypertension or diabetes leads to a loss of blood flow to the affected area, usually the legs [87]. One treatment goal for PAD is to increase vascular density to restore blood flow in the muscle. Experimentally, animal models of PAD are often generated via induction of hypertension by elevated salt intake or via ligation of a peripheral artery. In animal studies, antihypertensive treatment has generally been shown to decrease BP and increase vascular density peripherally, regardless of the model used [87]. These effects of antihypertensive medications have been shown to be dependent on AT₁R function in peripheral vessels, and AT₁R antagonism will inhibit reparative angiogenesis in ischaemic muscle. In animals with elevated BP, the antihypertensive prazosin was shown to increase angiogenesis in the rat anterior tibialis muscle, but this effect was abolished when animals were also given the ARB losartan [88]. Additionally, an earlier study by Munzenmaier and Greene [11] demonstrated that a subpressor dose of AngII increased blood vessel density in rat cremaster muscle and this increase was blocked by co-administration of losartan. Ischaemia models of PAD also support the anti-angiogenic character of ARBs, as AT₁R antagonism with candesartan resulted in the attenuation of ischaemia-induced angiogenesis in the mouse hindlimb [89]. Similar results were observed with losartan [90]. Following ischaemia induced by femoral artery occlusion in mice, aldosterone administration increased capillary density and blood flow, but this effect was blocked when aldosterone and valsartan were given in combination [91]. In the case of PAD, angiogenesis and re-vascularization of the affected muscle tissue is encouraged, and ARB treatment appears to inhibit these reparative processes. It is interesting that ARBs have been shown to interfere with angiogenic properties in both pathological conditions such as cancer and retinopathy as well as in reparative processes such as PAD. This dichotomy also exists within another system, the cardiovascular system, where ARBs have been shown to be either pro- or anti-angiogenic depending on the disease state under investigation.

Cardiovascular disease: experimental evidence

In a hamster model of cardiomyopathy and congestive heart failure, administration of valsartan throughout life was associated with a decrease in VEGF expression and a lower coronary capillary density [92]. This attenuation of coronary angiogenesis led to an increase in cardiac fibrosis, ultimately decreasing survival rates in animals. Interestingly, the ACEI enalapril had little effect on VEGF and improved survival rates in hamsters with cardiomyopathy [92]. Irbesartan was also shown to exert anti-angiogenic effects, blocking the pro-angiogenic actions of chronic intermittent hypoxia in a neonatal rat model of hypoxaemic congenital heart disease [93]. Treatment with the ARB candesartan was associated with decreased VEGF expression and decreased capillary density in a rat model of Type 2 diabetes [94]. In that study, candesartan administration was able to normalize the elevated capillary density found in diabetic hearts and improve cardiac structure to that observed in non-diabetic animals. Even in the normal heart, candesartan was shown to inhibit angiogenesis stimulated by direct VEGF application [95]. Although limited in number, these studies have indicated that ARBs could exert anti-angiogenic actions in the heart. Preventing or attenuating coronary angiogenesis could have deleterious effects on overall health, as in the hamster model of cardiomyopathy [92], or could improve cardiac function, as in animal models of diabetes [94].

PRO-ANGIOGENIC ACTIONS OF ARBs:

Cardiovascular disease: experimental evidence

The primary area in which ARBs appear to exert pro-angiogenic actions on cardiac tissue is during recovery from MI (myocardial infarction). Angiogenesis during post-MI myocardial remodelling can be a reparative process [96]. Inadequate blood supply following infarction can exacerbate infarct expansion and lead to pathological remodelling of both the infarcted and non-infarcted ventricle [97]. In an animal model of MI, losartan treatment was associated with increased coronary blood flow and increased capillary density in the non-infarcted left ventricle of rats [98,99]. Interestingly, this losartan-induced increase in microvessel density was subsequently found to be independent of VEGF expression [100]. The difference between pro- and anti-angiogenic effects of ARBs on cardiovascular disease appears to be dependent on the cellular environment: in non-acute cardiomyopathy models, valsartan, candesartan and irbesartan exhibited anti-angiogenic actions, but losartan actually promoted angiogenesis in animal models of MI.

Cardiovascular disease: clinical observations

In the ACCESS (Acute Candesartan Cilexetil thErapy in Stroke Survivors) trial, treatment with candesartan within 1 week after a stroke significantly reduced the number of vascular events (particularly MI) in patients up to 1 year after administration (9.8% in the candesartan arm and 18.7% in the placebo arm; $P = 0.026$) [101]. The MOSES (MORbidity and mortality after Stroke, Eprosartan compared with nitrendipine for Secondary prevention) trial also demonstrated significant protection from cardiovascular events (acute coronary syndrome, heart failure, fatal cardiac arrhythmia and pulmonary embolism) with eprosartan treatment [77 in the eprosartan arm and 101 in the nitrendipine arm; IDR (incidence density ratio), 0.75 (95% CI, 0.55–1.02); $P = 0.06$] [102].

Stroke: experimental evidence

Evidence supporting a pro-angiogenic effect of ARBs has come overwhelmingly from basic science studies of cerebral ischaemia or stroke (Table 1). Stroke occurs when blood flow to the brain is interrupted, creating a central area of cell death (the core) surrounded by compromised tissue (the penumbra). Although the core of the damaged tissue is often beyond repair, the penumbra can recover function if blood flow is restored in a timely manner. Human studies have demonstrated that increased angiogenesis in the penumbra region is correlated with increased survival in stroke patients [103]. Animal studies have confirmed the regenerative potential of penumbral vasculature and have been used to uncover novel ways to enhance adaptive angiogenesis in the injured brain [104]. Numerous studies have demonstrated that ARB treatment can reduce infarct size and improve behavioural recovery in stroke models in normal rats [105], spontaneously hypertensive rats [106] and in atherosclerotic ApoE (apolipoprotein E)-deficient mice [107].

Administration of losartan for 2 weeks prior to the initiation of cerebral ischaemia significantly increased the total area of vessels in the penumbra, primarily through an increase in average vessel size [13]. Interestingly, losartan treatment also increased blood vessel density in the absence of an ischaemic event, indicating that the pro-angiogenic effects of losartan could exist independently of traditional hypoxia-induced adaptations [13]. A subsequent study by the same group confirmed that losartan treatment for 2 weeks increased vascular density in the cortex in the absence of ischaemic insult [108]. Additionally, Li et al. [14] demonstrated that valsartan administration for 2 or 4 weeks increased cortical capillary density in mice prior to initiation of experimental stroke. In the same study, pre-treatment

with valsartan was also shown to increase cortical vascular density following ischaemia.

One mechanism by which ARBs could be acting is through hypotensive actions. However, animals given losartan for 2 weeks exhibited a small decrease in BP, which did not correlate well with the large increase in vascular density detected in the brains of treated animals. When the antihypertensive effect of losartan was mimicked with the ACEI captopril, no changes were observed in vascular density in the brain [108]. Another possible mechanism by which angiotensin receptor blockade could increase angiogenesis is by increasing AngII in circulation, which would then be free to interact with AT₂Rs. However, when cerebral blood vessel density was quantified following AngII infusion, it was found that losartan, but not AngII, increased vascular density [108]. One drawback of that study is that losartan administration increased circulating AngII approx. 5 times that of direct AngII infusion, limiting comparisons between the two groups [108]. Finally, work from our laboratory has demonstrated a pro-angiogenic effect of candesartan both *in vivo* and *in vitro* [15]. Having previously found that candesartan could improve outcome from experimentally induced stroke [109], we investigated the impact of candesartan on vascular density and angiogenic potential in both control animals and those undergoing MCAO (middle cerebral artery occlusion) to mimic cerebral ischaemia [15]. Animals given candesartan at the time of reperfusion had increased blood vessel density in the ischaemic hemisphere. CSF (cerebrospinal fluid) was harvested from animals at the time of killing and was then applied to brain microvascular endothelial cells, which responded by undergoing division, migration and transformation into tube-like structures resembling blood vessels *in vitro*. In line with other studies showing that ARBs could increase cerebrovascular density in the absence of injury [13], increased tube formation was found in cells treated with CSF from animals that had only received candesartan and had not undergone MCAO. This stimulation of angiogenesis was enhanced further in cells treated with CSF from stroke-injured animals [15].

In terms of a mechanism for ARBs inducing angiogenesis in brain endothelial cells, studies have investigated both direct effects of ARBs on cell signalling as well as the impact of shunting AngII to the AT₂R. Candesartan administration was associated with a decrease in the stress-related stathmin-like protein, FGF and tyrosine kinase receptor in brain microvessels [110]. Candesartan was also shown to reverse elevations in TNF α (tumour necrosis factor α), IL-1 β , NF- κ B (nuclear factor κ B) and the heat-shock proteins Hsp60, Hsp70 and Hsp90 in spontaneously hypertensive rats [111]. Telmisartan treatment prior to MCAO improved the number of neurons surviving ischaemia via decreased

expression of the cytotoxic PLA₂ (phospholipase A₂) in the infarcted brain [112]. Intracerebroventricular infusion of irbesartan in rats after MCAO resulted in a decrease in the expression of apoptotic proteins c-Fos and c-Jun in neurons [113]. In addition to reducing expression of cytotoxic mediators, ARB administration has also been demonstrated to potentially improve the regenerative capacity of the injured brain. Pre-treatment with candesartan (but not ramipril) prior to MCAO resulted in an increase in the brain expression of the TrkB receptor for BDNF (brain-derived growth factor), a potent protective and regenerating agent for neurons [114]. Considering that ARB treatment has been shown to increase AT₂R expression in the brain [115] and that the protective effect of candesartan on infarct volume was abolished when AT₂R and AT₄R (AngII type 4 receptors) were blocked [116], it appears that the mechanism of action of ARBs could involve the diversion of AngII away from AT₁R to interact with other AngII receptors. However, recent studies have indicated that ARBs could also exert direct effects on endothelial cells. High glucose exposure of brain-derived endothelial cells *in vitro* leads to nuclear translocation of the transcription factor NF- κ B; this translocation and subsequent expression of ICAM-1 and MCP-1 was prevented by treatment with both telmisartan and valsartan [80]. Interestingly, these results were observed in the absence of AngII, indicating an alternative mechanism of action of ARBs in endothelial cells.

Stroke: clinical observations

The majority of clinical trials investigating the impact of ARBs on stroke have focused on stroke prevention, not recovery. The MOSES trial demonstrated that eprosartan treatment significantly reduced the number of cerebrovascular events for up to 2 years following a transient ischaemic attack, stroke or intracerebral haemorrhage [102 for eprosartan compared with 134 for nitrendipine; IDR, 0.75 (95% CI, 0.58–0.97); $P = 0.026$] [102]. In the PRoFESS trial, telmisartan treatment for 2 years was associated with a non-significant reduction in stroke incidence [880 for telmisartan and 934 for placebo; HR, 0.95 (95% CI, 0.86–1.04)]. Similarly, in the stroke subgroup of ONTARGET, telmisartan exhibited a trend towards reducing stroke incidence (HR, 0.91 (95% CI 0.79–1.05)) [117]. A recent meta-analysis of ARBs in stroke prevention implied that, although ARBs exert a moderate protective effect against stroke, the benefit does not outweigh that observed with ACEIs or calcium antagonists [118]. Other studies, however, do postulate an increase in protection against stroke with ARBs compared with ACEIs when BP reduction is equalized in the analyses [119].

CONCLUSIONS

In summary, the effects of angiotensin receptors on angiogenesis vary according to disease state, animal model and specific medication investigated. Although the overwhelming weight of the evidence in basic science studies of cancer indicate that ARBs may inhibit tumour angiogenesis, studies in MI and cerebral ischaemia point to a potential pro-angiogenic role of ARBs. Whether this explains the increase in incidence of cancer in large clinical trials, as has been suggested by meta-analyses, remains debatable. The modulating effects of ARBs in angiogenic processes are likely to involve the growth factor VEGF as well as hypotensive responses and the availability of AngII for binding to other angiotensin receptors. However, each of these mechanisms alone cannot account for the multiplicity of actions of ARBs on the reduction or expansion of the existing vasculature network.

As ARBs have demonstrated safety and efficacy in randomized clinical trials and direct evidence of a cancer-causing effect is lacking, it is prudent to await further investigation before recommending a decrease in prescribing away from the ARBs. As basic science studies continue to unravel the exact effects of ARBs on angiogenesis in various disease models, more opportunities could actually develop for repurposing these medications for their positive vascular effects.

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