Angiotensin II-induced MMP-2 release from endothelial cells is mediated by TNF-α

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Submitted 17 September 2003; accepted in final form 25 November 2003

Arenas, Ivan A., Yi Xu, Patricio Lopez-Jaramillo, and Sandra T. Davidge. Angiotensin II-induced MMP-2 release from endothelial cells is mediated by TNF-α. Am J Physiol Cell Physiol 286: C779–C784, 2004.—Angiotensin II (ANG II) has been etiologically linked to vascular disease; however, its role in the alterations of endothelial function that occur in vascular disorders is not completely understood. Matrix metalloproteinases (MMPs) and proinflammatory cytokines are involved in the pathologic remodeling of blood vessels that occurs in vascular disease. In this study we evaluated the effects of ANG II on tumor necrosis factor (TNF)-α and MMP-2 production in endothelial cells. Human umbilical vein endothelial cells (HUVECs) were stimulated with ANG II (0.1–10 μM) for 24 h, in the presence or absence of antagonists of ANG II type 1 (AT1R) and type 2 (AT2R) receptors, and the production and release of TNF-α and MMP-2 were assessed. ANG II increased TNF-α mRNA and protein expression and the release of bioactive TNF-α. Moreover, ANG II induced MMP-2 release and reduced the secretion of tissue inhibitor of MMP (TIMP)-2 from endothelial cells. To elucidate whether endogenous TNF-α could mediate the effects of ANG II on MMP-2 release, cells were pretreated with anti-TNF-α neutralizing antibodies or pentoxifylline (an inhibitor of TNF-α synthesis). TNF-α inhibition prevented the secretion of MMP-2 induced by ANG II. Furthermore, AT1R antagonism with candesartan prevented the formation of MMP-2 and TNF-α, and the reduction of TIMP-2 induced by ANG II. These results indicate that ANG II, via AT1R, modulates the secretion of TNF-α and MMP-2 from endothelial cells and that TNF-α mediates the effects of ANG II on MMP-2 release.

remodeling; vasoactive mediators; inflammation

Angiotensin II (ANG II) is an important modulator of vascular homeostasis and an important link in the pathophysiology of cardiovascular disease (5, 27). Elevated ANG II and/or increased sensitivity to ANG II have been etiologically associated with major vascular diseases (5, 27). However, most of the studies concerning the effects of ANG II on vascular cells have been conducted in smooth muscle cells. An emerging role for ANG II is through modulation of endothelial cell function. Endothelial cells are essential to maintain normal vascular tone and blood fluidity and to limit vascular inflammation (29). Indeed, a common feature of vascular disorders is the presence of endothelial dysfunction.

Tumor necrosis factor (TNF-α) is proposed to be an important mediator of the endothelial alterations seen in vascular disease (10, 21). TNF-α-stimulated endothelial cells undergo functional alterations resulting in a prothrombotic and proinflammatory phenotype (activation) (20). TNF-α levels are elevated in a number of vascular disorders, and it appears to be involved in the chronic development of atherosclerosis as well as in the acute plaque events that can result in clinical events such as myocardial infarction or stroke (3, 30). TNF-α is principally derived from mononuclear phagocytes, but it can also be synthesized in vascular cells such as smooth muscle and endothelium (20). The role of TNF-α produced in vascular cells is not very well understood; however, it is likely to modulate key vascular processes such as angiogenesis and inflammation (10, 20, 21). Importantly, endothelium-derived TNF-α could contribute to the pathogenesis of vascular disease.

Interactions between ANG II and TNF-α may play an important role in the modulation of endothelial function. Some studies have suggested that TNF-α could mediate the vascular effects of ANG II (11). Interestingly, in endothelial cells, some of the effects of TNF-α, such as increased free radical production, inflammation, and enhanced remodeling, resemble those attributed to ANG II. Moreover, ANG II has been reported to activate proinflammatory transcription factors in endothelial cells known to induce the formation of TNF-α (6). Furthermore, ANG II has been shown to stimulate the production of TNF-α on other vascular cells (18). Altogether, these observations suggest that TNF-α could mediate some of the effects of ANG II on endothelial function.

Proinflammatory cytokines such as TNF-α have been shown to induce the release of matrix metalloproteinases (MMPs), including MMP-2 (14, 30). MMPs are a group of zinc-dependent endopeptidases that play a key role in matrix turnover. Indeed, increased interstitial matrix remodeling is believed to be involved in the pathogenesis of atherosclerosis and other vascular disorders (17, 24). MMP-2 participates in the breakdown of collagen type IV, a major component of subendothelial basement membrane (19, 28). Moreover, we previously reported (7, 8) that MMP-2, through cleavage of endothelium-derived peptides, may also lead to vasoconstriction and inflammation.

Enhanced MMP-2 activity has been shown to occur in vulnerable atherosclerotic plaques (1). Moreover, higher MMP-2 levels have been reported in patients after acute atherosclerotic events and in women with preeclampsia (22). All of these conditions have been associated with both increased effects of ANG II and higher levels of TNF-α. However, whether ANG II can induce the release of MMP-2 from
endothelial cells is unclear. In this study we evaluated the effects of ANG II on TNF and MMP-2 release from endothelial cells. We hypothesized that ANG II could induce the release of MMP-2 from endothelial cells, in part through the formation of TNF-α.

METHODS

All procedures were performed in conformance with the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society. Reagents. ANG II, PD-123319, and recombinant TNF-α were purchased from Sigma, whereas candesartan was obtained from Astra Pharma. Pentoxifylline (no. 002323, Hoechst Canada) was donated by Rabinovitch Pharma. Pentoxifylline (no. 002323, Hoechst Canada) was donated by ICN Biomedicals, and M199 medium, L-glutamine, and trypsin were purchased from Sigma, whereas candesartan was obtained from Astra American Physiological Society.

RESULTS

Effects of ANG II on MMP-2 and TIMP-2 release. To evaluate the effects of ANG II on MMP-2 and TIMP-2 release, HUVECs were stimulated with ANG II (0.1–10 μM) for 24 h. ANG II stimulation resulted in increased MMP-2 release (Fig. 1). Moreover, to determine the role of ANG II type 1 (AT₁R) and type 2 (AT₂R) receptors, cells were pretreated with antagonists of AT₁R (candesartan; 100 μM) or AT₂R (PD-123319; 100 μM) 1 h before ANG II stimulation. Pretreatment with candesartan, but not with PD-123319, prevented the secretion of MMP-2 induced by ANG II (Fig. 2). In similar cell culture conditions, ANG II did not affect the secretion of MMP-9 (92 kDa). Furthermore, ANG II reduced the secretion of TIMP-2 from endothelial cells, which was also inhibited by AT₁R.
antagonism (Fig. 3). AT$_2$R antagonism did not affect ANG II-induced TIMP-2 release.

**Effects of ANG II on TNF-α formation.** TNF-α is first synthesized as an immature peptide (pro-TNF-α) that is later cleaved by a TNF-α-converting enzyme (20). After treatment with ANG II (0.1–10 μM) for 24 h, TNF-α protein was evaluated in cell lysates with an antibody able to detect mature and immature forms of TNF-α. We found pro-TNF-α and mature TNF-α protein levels to be significantly higher in cells stimulated with ANG II compared with control (Fig. 4). The effect of ANG II on TNF-α mRNA transcription was evaluated by real-time RT-PCR. RNA isolated from control and ANG II-treated cells was reverse transcribed, and first-strand cDNA was further amplified. The average threshold for ANG II-
Effects of TNF-α inhibition on ANG II-induced MMP-2 release. To evaluate whether TNF-α can mediate the effect of ANG II on MMP-2 release, cells were first pretreated with neutralizing antibodies against human TNF-α 1 h before ANG II stimulation. The concentration of anti-TNF-α antiserum was calculated based on the ability to neutralize the effects of 50 pg of TNF-α on L929 cells. TNF-α blockade significantly reduced (P < 0.05) the secretion of MMP-2 induced by ANG II (Fig. 7). The use of antiserum alone did not affect the basal release of MMP-2. Moreover, pretreatment with pentoxifylline (0.1 and 1 mg/ml), a nonselective phosphodiesterase inhibitor that has been shown to inhibit TNF-α synthesis, also prevented the release of MMP-2 induced by ANG II (reduction of 40 ± 10% and 110 ± 9%, respectively, compared with ANG II alone; P < 0.05). Pentoxifylline (1 mg/ml) did not significantly change the basal release of MMP-2 from endothelial cells.

DISCUSSION

Our data indicate that ANG II-induced release of endothelial MMP-2 is mediated by TNF-α. It has been shown that ANG II may induce a proinflammatory phenotype in endothelial cells (i.e., increase the expression of adhesion molecules) (25). However, to our knowledge, there are no previous studies reporting the effects of ANG II on TNF-α formation in en-
The present study shows evidence of TNF-α generation at the levels of mRNA, protein expression, and function. These observations are clinically relevant because of the key role of ANG II and TNF-α in the pathogenesis of vascular disorders such as preclampsia and atherosclerosis and the availability of treatments to antagonize these factors.

In fact, recent studies have shown that TNF-α antagonism improves endothelial function in patients with chronic heart failure (12) or chronic inflammation (15). Although the concentration of ANG II that results in a maximum effect on TNF-α production is higher than the reported circulating levels, it is likely that circulating levels underestimate the concentration available in vascular beds. For instance, there is evidence that locally formed (within vascular walls) ANG II may account for part of its vascular effects (2).

The fact that ANG II may induce the formation of active TNF-α in endothelial cells is intriguing. ANG II induced TNF-α gene expression as well as the formation of immature and bioactive forms of TNF-α, which suggests that ANG II stimulates the proteolytic cleavage of pro-TNF-α. TNF-α is an inflammatory cytokine proposed to be a mediator of the endothelial alterations seen in cardiovascular disease (10, 20). Indeed, TNF-α has been shown to induce endothelial dysfunction, inflammation, and apoptosis (10, 20, 21). Interestingly, some of the effects of TNF-α on endothelial function, such as increased free radical production, inflammation, and enhanced remodeling, resemble those attributed to ANG II. On the other hand, TNF-α has been shown to protect endothelial cells from apoptosis by inducing platelet-derived growth factor pathways (13). Therefore, endogenous production of TNF-α could trigger either detrimental or protective pathways to modulate the effects of ANG II on endothelial cells. Understanding the role of interactions between ANG II and TNF-α on endothelial modulation of vascular function is necessary.

MMP-2 is an important protease involved in normal (angiogenesis or wound repair) and pathological (chronic inflammation and tumor growth) blood vessel remodeling (19, 28). In this study, we found that ANG II increases the release of MMP-2 from endothelial cells while decreasing the secretion of TIMP-2, the endogenous inhibitor of MMP-2, which was prevented by AT,R antagonism. Therefore, the increased MMP-2 activity after ANG II stimulation could be due to both increased MMP-2 release and decreased TIMP-2 secretion. These observations may indicate that ANG II, via AT,R, could induce matrix turnover by enhancing the release activity of MMP-2.

Interestingly, opposing findings have been reported in vascular smooth muscle cells and fibroblasts, in which ANG II decreased the secretion of MMP-2 (23, 26). These observations suggest a cell-specific effect of ANG II, which is important to understand when using AT,R antagonists clinically. It could be speculated that for smooth muscle cells and fibroblasts ANG II-mediated decrease in MMP-2 release could lead to collagen deposition promoting fibrosis, whereas in endothelial cells ANG II-induced MMP-2 activity could be involved in other processes such as angiogenesis, thrombosis, and inflammation. Indeed, we have shown (7, 9) that MMP-2, through cleavage of endothelium-derived peptides, can directly promote vasoconstriction and facilitate leukocyte recruitment.

The present study suggests that a dysfunctional endothelium caused by ANG II may directly affect the development and progression of atherosclerosis and other vascular disorders by enhancing the local production of TNF-α, a potent inflammatory cytokine postulated to be an important mediator of vascular disease. Although in most vascular disorders upregulation of ANG II, TNF-α, and MMP-2 have been described, the contribution of TNF-α and MMP-2 to the pathophysiological effects of ANG II remains to be understood. Furthermore, these findings also suggest that MMP-2 and/or TNF-α inhibition could have potential therapeutic implications in some vascular disorders in which ANG II plays a role in the pathogenesis of vascular dysfunction.

ACKNOWLEDGMENTS

We gratefully acknowledge Dr. Sheena Fang for technical assistance and Dr. Larry Guilbert for helpful suggestions.

GRANTS

The Canadian Institute for Health Research supported this study. S. T. Davidge is a Canada Research Chair in Women’s Cardiovascular Health and an Alberta Heritage Foundation for Medical Research (AHFMR) Senior Scholar. Ivan A. Arenas is an AHFMR scholarship recipient and a fellow in Tomorrow’s Research Cardiovascular Health Professionals (TORCH).

REFERENCES


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