Erk MAPK Activation and Cellular Proliferation at the Human Carotid Bifurcation is Prevented by ACE-Inhibitor Therapy

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Citation

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Abstract

Objective: We observed extracellular signaling-regulated kinase 1/2 (Erk1/2)-mitogen activated protein kinase (MAPK) activation in vascular smooth muscle-like cells in response to cyclic mechanical strain, and this mediated proliferation and matrix elaboration. Thus, we asked whether chronic Erk activation might occur at a site of ongoing mechanical strain in the human carotid bulb, whether this was related to Angiotensin II and if such activation were associated with cellular proliferation.

Approach: Human carotid arteries (n=69) were collected at the time of endarterectomy en bloc from intima to external elastic lamina. Two sections were taken from above, at, and below the bifurcation. One was placed in liquid nitrogen for protein extraction and one into formalin for immunostaining. Clinical data were collected anonymously into a database. Twelve samples (6 from subjects taking ACE inhibitors (ACE-I) and 6 without ACE-I therapy) form the basis of this report. Protein from each section was extracted and analysed by western blotting for Erk activation and proliferating cell nuclear antigen (PCNA) expression. Localization of active (phosphorylated) Erk was assessed by immunostaining.

Results: The strongest Erk activation was observed at and above the bifurcation, with little activation in the common carotid. Expression of PCNA mirrored Erk activation. Immunostaining revealed Erk activation primarily in the medial layer. Therapy with ACE inhibitors largely prevented both Erk activation and PCNA expression.

Conclusion: Chronic Erk activation is seen at the human carotid bulb, and is associated with PCNA expression. The presence of ACE-I prevents these changes.

INTRODUCTION

Atherosclerosis is the result of a process of remodelling of the arterial wall caused by exposure to physical of chemical noxious agents. In this process a myriad of molecular reactions is involved, mostly unknown, but from those known, the complexity of metabolic pathways leading to atherosclerosis is clear. In recent years progress has ben made in the understanding of molecular reactions involved in initiating and leading to the progression of atherosclerosis. However, studies on atherogenesis to be believable and reproducible need to be conducted in arterial specimens, which present the full spectrum of the atherosclerotic lesions, from normal artery, to early lesions, advanced, unstable and complex lesions with ulceration and necrosis. Moreover, the specimens need to originate from a specific artery (since arteries in different districts may have different structural characteristics) and must have only one origin (either form cadaver or from a surgical specimen). Carotid arteries appear to be ideal for this type of studies and the atherosclerotic disease has a strong tendency to occur at their bifurcation;[1] turbulence of blood flow leading to changes in wall stresses and decreased oscillatory shear are thought to play a critical role.[2] The bifurcation or carotid bulb is the region where circumferential tension (pressure pulses) and wall shear stress are most out of phase,[3] and the pulsatile pressure has recently been shown to correspond best with increases in intima-media thickness.[4]

Extracellular signal-regulated kinase (Erk) mitogen activated protein kinase (MAPK) is activated in response to cyclic

mechanical stress in vascular smooth muscle cells (VSMC) in vitro5 and in response to acute increases in mechanical stress in rat carotid arteries in vivo.[6] Stretch in an isolated porcine carotid artery model was also seen to lead to Erk activation.[7] Erk activation in atherosclerotic lesions from hypercholesterolemic rabbits was primarily seen in smooth muscle cells and correlated with proliferation both in vivo and in vitro.[8] Downstream Erk-dependent activation of the transcription factor AP-1 has been demonstrated in rat carotids exposed to pressure loads, and hypothesized to lead to VSMC hyperplasia and matrix protein elaboration.[6] The hyperplasia suppressor gene mitofusin-2 inhibits lesion development in spontaneously hypertensive rat and apo-E knockout mouse carotids via Erk inhibition.[9]

Angiotensin II (AII), generated locally by angiotensin-converting enzyme (ACE) plays a central role in vascular remodelling and atherosclerosis.[10] The primary pathologic effects of AII are mediated through the AT1 receptor, which is linked downstream to Erk, VSMC hypertrophy/hyperplasia and matrix protein elaboration.[11] In spontaneously hypertensive rats, ACE inhibitors (ACE-I) reduced Erk activity in the vascular wall and, conversely, Erk inhibition prevented AII-induced arterial contractility.[12] ACE is expressed in atherosclerotic lesions, including the human carotid, both in macrophage foam cells and VSMC.[13,14] In mature human carotid atherosclerotic lesions, ACE expression in the lesion shoulder region was proposed to contribute to high levels of AII in this area, inflammation and plaque instability.[13] Similarly, ACE activity in human coronary vessels obtained at atherectomy was higher in patients with acute coronary syndrome as opposed to stable disease.[15] ACE inhibition retards atherosclerotic lesion development in numerous animal models, and inhibits Erk activation in balloon-injured rat carotid artery. [10,16] The ability of ACE-I to prevent vascular outcome events in humans is less clear, with large trials showing either a beneficial[17,18] or no effect.[19] It is hypothesized that a minimum level of vascular risk must exist for a benefit to be shown.

We thus sought to determine whether Erk activation and cellular proliferation could be observed in mature human carotid artery lesions, and whether such activation was primarily observed in the lesion or the medial (VSMC) layer. We further sought to determine if ACE-I had an effect on ERK activation and cellular proliferation.

MATERIALS AND METHODS

Subjects and creation of a carotid endarterectomy tissue bank. Since Erk MAPK has been demonstrated to be activated by mechanical forces by ourselves[20] and others in numerous tissues including VSMC[21], we sought a model that would permit assessment of whether this observation held in human vasculature. We chose the carotid artery, since tissue is commonly available, and the endarterectomy "cast" removed at surgery contains all layers internal to the external elastic lamina. Importantly, disease tends to occur in a very stereotyped fashion in the carotid bulb at, and above, the bifurcation, with the small portion of arterial "cast" proximal and distal to the bifurcation appearing grossly uninvolved.

Patients in whom carotid endarterectomy was planned for symptomatic[22] or asymptomatic[23] carotid stenosis were consented at their initial surgical office visit. Demographic data, presence of ipsilateral symptoms, degree of stenosis, medical history, medication use, blood pressure and laboratory data were obtained. At surgery, carotid endarterectomy was performed along the external elastic lamina and the plaque removed en bloc from below to above the bifurcation, to include the entire atherosclerotic lesion. Two transverse sections were taken from the distal internal carotid above (level D) and common carotid below the bifurcation (level A), and at the bifurcation itself (level C) (Figure 1). One section at each level was divided into 3 pieces and placed immediately into liquid nitrogen for later protein extraction and western blotting; the other section at each level was placed into formalin and then paraffinized.

Protein extraction and western blotting. Carotid tissue was placed into a mortar containing a small amount of liquid nitrogen to which was added 250-500µl (at10 times the wet weight of the tissue) of homogenizing buffer (50mM Tris pH7.4, 150mM NaCl, 5mM EDTA, 1% Triton X-100, 10% glycerol, 1mM sodium fluoride, 1mM Beta-glycerophophate, 0.1mM sodium vanadate, 60mM N-octyl glucopyronisde, 2µl/ml aprotinin, 2µl/ml leupeptin, 1mM PMSF). The frozen tissue was ground in buffer until a fine powder was obtained. This powder was processed in a dounce homogenizer until transformed into liquid (20-30 strokes). The liquid was transferred to a precooled 1.5 ml tube, incubated on ice for 30 minutes and then centrifuged at 14,000 rpm for 10 minutes at 4 C°. The supernatant (50µg) was separated on 10% SDS-PAGE, and Western blotting performed, as we have described.[24] Briefly, after SDS-PAGE separation and electroblotting to a nitrocellulose membrane

(Amersham, Baie d'Urfe, Quebec, Canada), membranes were blocked for 1 hour at room temperature in blocking buffer (Tris-buffered saline with 0.1% Tween-20 (TTBS) and 5% w/v non-fat dry milk), and then overnight with gentle rocking at 4 C° with primary antibody (in TTBS with 5% BSA). Membranes were then washed 3 times with TTBS and incubated with HRP-conjugated secondary antibody (1:5000, BioRad, Mississauga, Ontario, Canada) in blocking buffer for 1 hour at room temperature. After 3 further washes, the membrane was incubated with ECL (Amersham) and then exposed to X-ray film (X-OMAT). Antisera used included polyclonal phospho-p44/42 MAP Kinase (Thr202/Tyr204) (1:1000, for activated Erk, Cell Signaling, Beverly MA), polyclonal p44/42 MAP Kinase (1:1000, Cell Signaling), monoclonal PCNA (PC10)(1:1000, for cellular proliferation, Cell Signalling) and monoclonal Beta-actin (to ensure equal protein loading, Sigma-Aldrich, Oakville, Canada, 1:5000).

Immunostaining. The tissue was processed initially into paraffin. Before staining, it was sectioned at 4 micron and deparaffinized. Endogenous peroxidase was blocked with H2O2 in methanol. After blocking with normal goat serum, sections were incubated with polyclonal phospho-p44/42 MAP Kinase (Thr202/Tyr204) at 1:100, then with biotinylated secondary, streptavidin-HRP and Nova Red chromogen. Slides were then counterstained with hematoxylin, dehydrated and mounted in Permount. Slides were visualized using an Olympus microscope. Positive nuclei were counted from sections below the bifurcation (level A in Figure 1) and at the bifurcation/bulb (level C in Figure 1). Ten fields at 200X were counted for each sample at each level by an investigator (AI) blinded to treatment (ACE-I versus no ACEI).

RESULTS

Erk MAPK activation occurs at the carotid bulb and is inhibited by ACE-I therapy. To date we have assembled a bank of 69 carotid artery samples with clinical information. Of these, only 6 patients were not receiving ACE-I therapy at the time of surgery. Consequently, we chose these 6 patients and 6 controls carefully matched for age, sex, history of vascular events, presence of diabetes or hypertension and concomitant cardiac medications (beta blockers, calcium channel blockers, antiplatelet agent and HMG CoA reductase inhibitors).

Western blot analysis (Figure 2 A, B) showed that Erk activation in patients not treated with ACE-I was most prominent in the bulb at the bifurcation and at the internal carotid above (levels C and D in Figure 1). Densitometry (Figure 3) shows that the difference was significant at $p<0.05$ between level C and D relatively to the control level A and between specimens from patients not treated and treated with ACE-I.

Cell proliferation occurs at the carotid bulb and is inhibited by ACE-I therapy. We sought to determine whether cell proliferation, as assessed via western blotting for PCNA, mirrored Erk activation and if proliferation was affected by the presence of ACE-I. Indeed, PCNA expression was seen primarily at levels corresponding to the carotid bulb (levels C and D in Figure 1) in patients not treated with ACE-I; little expression was seen in the common carotid at level A (Figure 4). Expression was markedly increased in the bulb and was 3-5 fold greater than observed in the common carotid. Figure 4 shows that the presence of ACE-I prevented the increased PCNA expression in the human carotid bulb to the point where it was not significantly different than the common carotid artery below.

Erk activation occurs primarily in the smooth muscle layer. We sought to confirm that the increased Erk activation we observed at levels through the carotid bulb was primarily in the VSMC (medial) layer, rather than in the lesions themselves. Carotid arterial sections were stained for phosphorylated (active) Erk and examined microscopically. Ten high power (200X) fields were examined from each subject. Figure 5 panels A through C shows representative sections at 200X. Erk nuclear staining is seen almost entirely in the bulb and in the medial layer (Panel C). No staining was seen without primary antibody (Panel B), and very little was seen at the common carotid below the bifurcation (Panel A). Positive nuclei in 10 random high power fields were counted by an investigator blinded to the presence or absence of ACE-I (Panel 5D). Positive staining was largely prevented by the presence of ACE-I (Figure 5D).

Figure 1

Human Carotid Artery at Endarterectomy. Schematic of a human carotid cast as it is removed at endarterectomy. The usual site and extent of gross atherosclerotic lesions are shown. Samples were taken at the levels indicated (CC= Common Carotid Artery; EC = External Carotid Artery; IC = Internal Carotid artery)

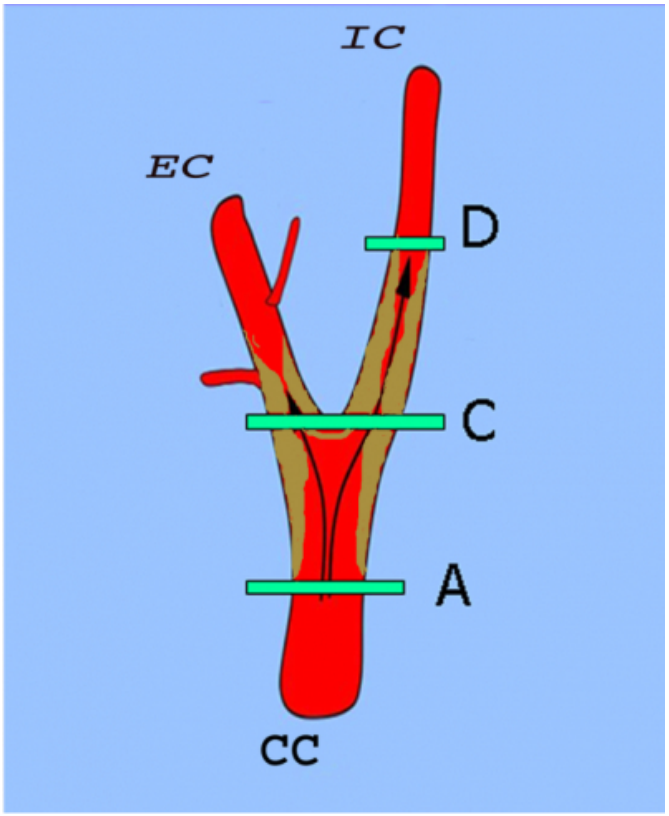


Figure 2

Erk Activation Occurs in the Carotid Bulb and is Inhibited in the Presence of ACE-I. A) Erk activation as assessed by western blotting of human carotid tissue is increased in the carotid bulb, but not in the common carotid below. B) ACE-I therapy prevents this increase in Erk activation.

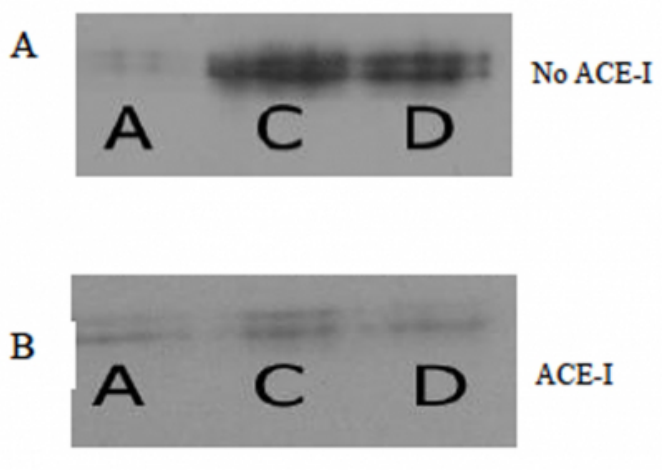


Figure 3

Data relative to Erk Activation, in patients treated with ACE inhibitors and non (n=6 per condition) are shown densitometrically (*indicates p<0.05 versus the corresponding control level A).

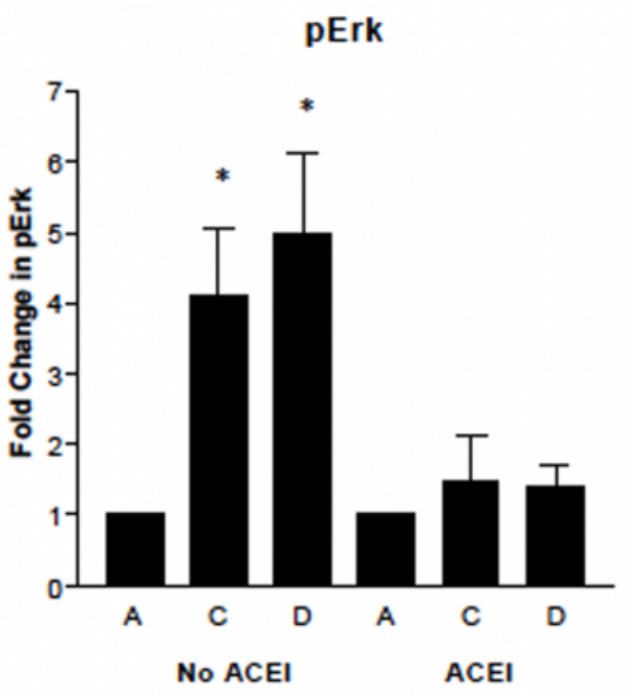


Figure 4

Cell Proliferation Occurs in the Carotid Bulb and is Inhibited in the Presence of ACE-I. A) PCNA expression as assessed by western blotting of human carotid tissue is increased in the carotid bulb, but not in the common carotid below. B) ACE-I therapy prevents this increase in PCNA expression. C) Data (n=6 per condition) are shown densitometrically (*indicates p<0.05 versus the corresponding control level A).

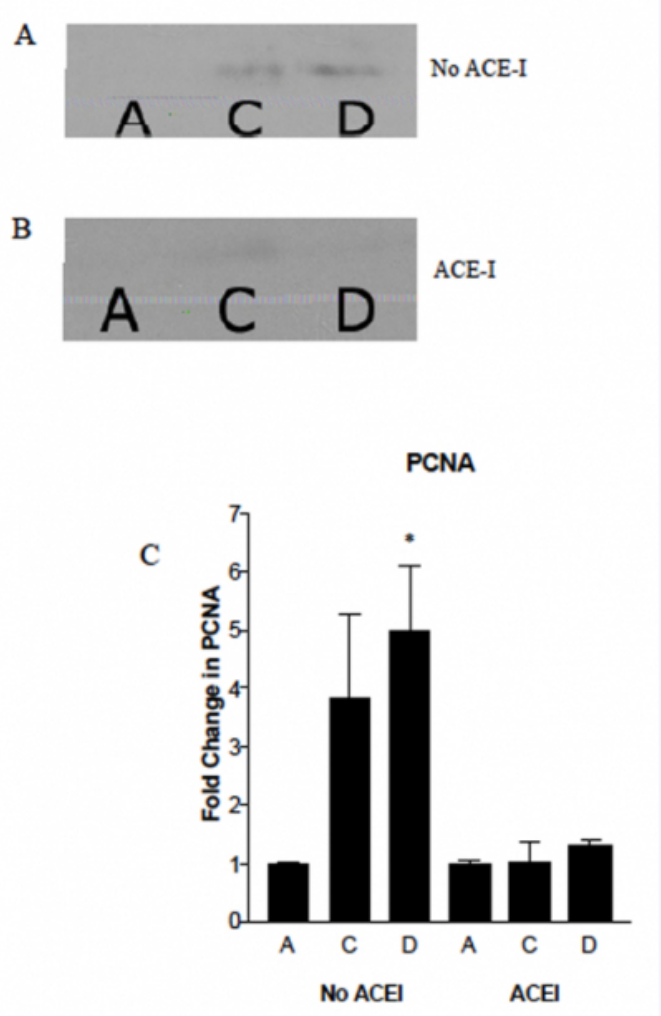
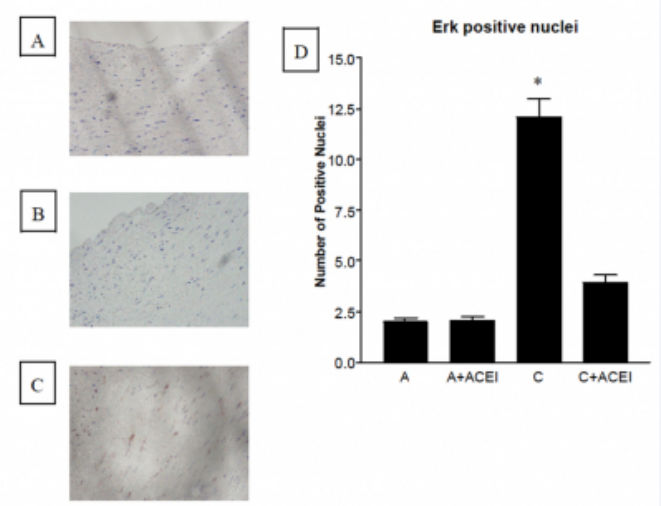


Figure 5

Erk Activation Occurs Primarily in the Smooth Muscle Layer. A) Very infrequent Erk positive nuclei were seen below the bifurcation in the common carotid. B) No positive staining was seen in the absence of primary antibody. C) Numerous positive nuclei, largely in the medial layer, were seen at the bifurcation. D) Ten high power (200X) fields were examined from each subject (n=6 per condition) and counted by an observer blinded to presence or absence of ACE-I. The presence of ACE-I significantly decreased staining at the bifurcation (* p<0.05).



DISCUSSION

This work shoes that Erk activation and cell proliferation occurs at the carotid bulb, and primarily in the medial layer in VSMC. For the first time, we also demonstrate that in humans the presence of ACE-I can largely abrogate both Erk activation and cell proliferation. The data presented here appear in line with those in the literature.

Modeling studies using computational fluid dynamics applied to flow data from 3D ultrasound indicate that increased tensile pressure is the strongest predictor of intimal-medial thickness in the carotid bulb,[4] and that, in patients with stenosis, the turbulent flow and low wall shear stress that are thought to be most atherogenic, occur distal to the stenosis (in the internal carotid) and are proportional to the severity of the stenosis.[25] Moreover, it has been shown that elevated ACE levels may be seen in mature human carotid atherosclerotic shoulder regions,[13] that AII may lead to activation of Erk both in vitro and in animal arteries in vivo,[11,12] and that ACE inhibition prevents Erk activation in the vascular wall in animal models.[12] Proliferation of VSMC is a hallmark of the pathogenesis and development of atherosclerosis.[26] Erk MAPK is responsive to mechanical

stresses in vascular VSMC in vitro[5] and in rat and pig carotid arteries in vivo.[6,7] Mechanical stresses stimulate cell proliferation, and Erk-dependent activation of the transcription factor AP-1 has been demonstrated to be central to this in numerous in vitro and in vivo systems. This includes, most relevant to this work, rat carotids exposed to pressure loads.[6] Interestingly, pulsatile mechanical stresses promote ACE expression in human aortic smooth muscle cells,[22] indicating that perhaps greater local generation of AII could occur at specific vascular areas in response to altered mechanical forces, as it has been suggested by others.[23] AII is generated locally in the vasculature by ACE.[10] AII signaling through the AT1 receptor has been shown to stimulate Erk activation and VSMC proliferation in vitro.[11] Therapy with ACE-I reduces Erk activity in spontaneously hypertensive rat vasculature.[12] In the diseased human carotid, ACE mRNA and protein expression is seen primarily in the intima, but is also enriched in macrophages and VSMC in the shoulder region.[13] There is data to suggest that the presence of ACE-I in humans can reduce inflammation in the carotid plaque removed at endarterectomy, as measured by decreased NFkappaB and C-reactive protein expression.[24] It should be noted, however, that the ability of AII to induce scar collagen production in mouse aorta was Erk dependent but NfkappaB independent,[25] and the stress activated protein kinases jun-N terminal kinase and p38 MAPK are classically thought of as upstream of NfkappaB signaling, rather than Erk.[26]

The carotid artery presents a unique vascular bed in which to study the role of mechanical forces in the activation of Erk in the human vasculature. Carotid atherosclerotic disease occurs in a very stereotyped fashion, with lesions forming in the bulb (at the bifurcation and just above in the internal carotid artery).[1] There has been considerable biophysical work using fluid dynamics and the anatomy of the carotid at this location to ascertain why atheroma tend to form at the bulb; initial studies were based on assumptions of Poiseuille flow and implicated primarily decreased wall shear stress.[2] However, the assumption of Poiseuille flow (which assumes a constant circular cross-section) in already diseased arteries is not appropriate, and more recent studies implicate increased pressure as the primary determinant of wall thickness,[27] and suggest that areas where circumferential tension and shear stress are most out of phase are at greatest risk of atheroma formation.[4,28] These conditions exist at the carotid bulb.[3] The carotid also provides a useful model since it is removed en bloc at operation, including the medial layer, from the common carotid to above the bulb. Thus, grossly normal control artery (the common carotid) and diseased artery (the bulb) are present in the same sample.

The logical assumption from these and our data is that inhibition of the renin angiotensin system would help prevent formation of carotid atheroma and therefore help prevent strokes. When one considers patients with known vascular disease, prevention of all vascular events (including stroke) by ACE-I therapy is controversial,[17-19] but the general consensus of opinion is that ACE-I are effective if the baseline risk is sufficiently high. Trials of primary prevention of stroke with renin-angiotensin interruption have been largely positive,[29,30] although controversy still exists as to whether specific protection derived from prevention of angiotensin II generation or signaling can be teased out from blood pressure lowering, which is clearly the most effective primary prevention strategy.[31] More recently, however, angiotensin receptor blockade (ARB) therapy was shown to be ineffective in preventing second strokes when started promptly after a first stroke.[32] Thus, the clinical data would suggest that renin-angiotensin inhibition might be most effective in prevention of initial formation of atheroma, rather than stabilization of mature lesions. Nevertheless, our data does indicate a biologic effect of the presence of ACE-I even in mature lesions, suggesting that we have yet more to learn about the biology and potential therapies for advanced carotid atherosclerotic disease. In conclusion, the data presented here demonstrate Erk activation and cell proliferation in the human carotid bulb and show for the first time that this can be largely abrogated by ACE-I therapy. This provides some mechanistic insight into how renin-angiotensin interruption may help to prevent strokes.

HIGHLIGHTS

- In human carotid arteries collected at the time of endarterectomy, chronic extracellular signaling-regulated kinase (ERK) activation occurs at and above the carotid bifurcation, and little in the common carotid.
- Expression of proliferating cell nuclear antigen (PCNA) mirrored Erk activation.
- Immunostaining revealed that Erk activation occurs primarily in the medial layer. Therapy with ACE inhibitors largely prevented both Erk activation and PCNA expression

References

1. Zarins CK, Giddens DP, Bharadvaj BK, Sottiurai VS, Mabon RF, Glagov S. Carotid bifurcation atherosclerosis. Quantitative correlation of plaque localization with flow velocity profiles and wall shear stress. Circ Res. 1983:53:502-514.

2. Ku DN, Giddens DP, Zarin CK, Glagov S. Pulsatile flow and atherosclerosis in the human carotid bifurcation. Positive correlation between plaque location and low oscillating shear stress. Arteriosclerosis 1985:5:293-302.
3. Tada S, Tarbell JM. A computational study of flow in a compliant carotid bifurcation-stress phase angle correlation with shear stress. Ann Biomed Eng. 2005:33:1202-1212.
4. Augst AD, Ariff B, McG Thom SA, Xu XY, Hughes AD. Analysis of complex flow and the relationship between blood pressure, wall shear stress, and intima-media thickness in the human carotid artery. Am J Physiol Heart Circ Physiol. 2007:293: H1031-H1037.
5. Li C, Hu Y, Mayr M, Xu Q. Cyclic strain stress-induced mitogen-activated protein kinase (MAPK) phosphatase 1 expression in vascular smooth muscle cells is regulated by Ras/Rac-MAPK pathways. J Biol Chem. 1999:274:25273-25280.
6. Xu Q, Liu Y, Gorospe M, Udelsman R, Holbrook NJ. Acute hypertension activates mitogen-activated protein kinases in arterial wall. J Clin Invest. 1996:97:508-514.
7. Franklin MT, Wang CL, Adam LP. Stretch-dependent activation and desensitization of mitogen-activated protein kinase in carotid arteries. Am J Physiol. 1997:273:C1819-C1827.
8. Hu Y, Dietrich H, Metzler B, Wick G, Xu Q. Hyperexpression and activation of extracellular signal-regulated kinases (ERK1/2) in atherosclerotic lesions of cholesterol-fed rabbits. Arterioscler Thromb Vasc Biol. 2000:20:18-26.
9. Chen KH, Guo X, Ma D, Guo Y, Li Q, Yang D, Li P, Qiu X, Wen S, Xiao RP, Tang J. Hyperexpression and activation of extracellular signal-regulated kinases (ERK1/2) in atherosclerotic lesions of cholesterol-fed rabbits. Nat Cell Biol. 2004:6:872-883.
10. Heeneman S, Sluimer JC, Daemen MJ. Angiotensin-converting enzyme and vascular remodeling. Circ Res. 2007:101:441-454.
11. Ohtsu H, Suzuki H, Nakashima H, Dhobale S, Frank GD, Motley ED, Eguchi S. Angiotensin II signal transduction through small GTP-binding proteins: mechanism and significance in vascular smooth muscle cells. Hypertension. 2006:48:534-540.
12. Touyz RM, Deschepper C, Park JB, He G, Chen X, Neves MF, Virdis A, Schiffrin EL. Inhibition of mitogen-activated protein/extracellular signal-regulated kinase improves endothelial function and attenuates Ang II-induced contractility of mesenteric resistance arteries from spontaneously hypertensive rats. J Hypertens. 2002:20:1127-1134.
13. Fukuhara M, Geary RL, Diz DI, Gallagher PE, Wilson JA, Glazier SS, Dean RH, Ferrario CM. Angiotensin-converting enzyme expression in human carotid artery atherosclerosis. Hypertension. 2000:35:353-359.
14. Ribichini F, Pugno F, Ferrero V, Bussolati G, Feola M, Russo P, Di Mario C, Colombo A, Vassanelli C. Cellular immunostaining of angiotensin-converting enzyme in human coronary atherosclerotic plaques. J Am Coll Cardiol. 2006:47:1143-1149.
15. Hoshida S, Kato J, Nishino M, Egami Y, Takeda T, Kawabata M, Tanouchi J, Yamada Y, Kamada T. Increased angiotensin-converting enzyme activity in coronary artery specimens from patients with acute coronary syndrome. Circulation 2001:103:630-633.
16. Kim S, Izumi Y, Yano M, Hamaguchi A, Miura K, Yamanaka S, Miyazaki H, Iwao H. Angiotensin blockade inhibits activation of mitogen-activated protein kinases in rat balloon-injured artery. Circulation. 1998:97:1731-1737.
17. Fox KM. Efficacy of perindopril in reduction of cardiovascular events among patients with stable coronary artery disease: randomised, double-blind, placebo-controlled, multicentre trial (the EUROPA study). Lancet. 2003:362:782-788.
18. Yusuf S, Sleight P, Pogue J, Bosch J, Davies R, Dagenais G. Renin Angiotensin Aldosterone System Inhibitors In Hypertension: Is There Evidence For Benefit Independent Of Blood Pressure Reduction? N Engl J Med. 2000:342:145-153.
19 Braunwald E, Domanski MJ, Fowler SE, Geller NL, Gersh BJ, Hsia J, Pfeffer MA, Rice MM, Rosenberg YD, Rouleau JL. Angiotensin-converting-enzyme inhibition in stable coronary artery disease. N Engl J Med. 2004:351:2058-2068.
20. Ingram AJ, Ly H, Thai K, Kang M, Scholey JW Activation of mesangial cell signaling cascades in response to mechanical strain. Kidney Int. 1999:55:476-485.
21. Li C, Hu Y, Mayr M, Xu Q. Cyclic strain stress-induced mitogen-activated protein kinase (MAPK) phosphatase 1 expression in vascular smooth muscle cells is regulated by Ras/Rac-MAPK pathways. J Biol Chem. 1999:274:25273-25280.
22. Cina C S, Clase CM, Haynes RB. Carotid endarterectomy for symptomatic carotid stenosis. Cochrane Database Syst Rev. 2000:2:CD001081.
23. Chambers BR, Donnan GA. Carotid endarterectomy for asymptomatic carotid stenosis. Cochrane Database Syst Rev. 2005:4:CD001923.
24. Krepinsky JC, Li Y, Chang Y, Liu L, Peng F, Wu D, Tang D, Scholey J, Ingram AJ. Akt mediates mechanical strain-induced collagen production by mesangial cells. J Am Soc Nephrol. 2005:16(6):1661-72.
20. 25. Xue YJ, Gao PY, Duan Q, Lin Y, Dai CB. Preliminary study of hemodynamic distribution in patient-specific stenotic carotid bifurcation by image-based computational fluid dynamics. Acta Radiol. 2008:49:558-565.
21. 26. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. Nature. 1993:362(6423):801-809.
22. 27. Iizuka K, Machida T, Kawaguchi H, Hirafuji M. Pulsatile mechanical pressure promotes Angiotensin-converting enzyme expression in aortic smooth muscle cells. Cardiovasc Drugs Ther. 2008:22:383-390.
23. 28. Mehta PK, Griendling KK. Angiotensin II cell signaling: physiological and pathological effects in the cardiovascular system. Am J Physiol Cell Physiol. 2007:292:C82-C97.
24. Sattler KJ, Woodrum JE, Galili O, Olson M, Samee S, Meyer FB, Zhu XY, Lerman LO, Lerman A. Concurrent treatment with renin-angiotensin system blockers and acetylsalicylic acid reduces nuclear factor kappaB activation and C-reactive protein expression in human carotid artery plaques. Stroke. 2005:36, 14-20.
25. Tharaux PL, Chatziantoniou C, Fakhouri F, Dussaule JC. Angiotensin II activates collagen I gene through a mechanism involving the MAP/ER kinase pathway. Hypertension. 2000:36: 330-336.
26. Ellinger-Ziegelbauer H, Brown K, Kelly K, Siebenlist U. Direct activation of the stress-activated protein kinase (SAPK) and extracellular signal-regulated protein kinase (ERK) pathways by an inducible mitogen-activated protein Kinase/ERK kinase kinase 3 (MEKK) derivative. J Biol Chem. 1997:272: 2668-2674.
27. Laurent S, Tropeano AI, Lillo-Lelouet A, Jondeau G, Laloux B, Boutouyrie P. Local pulse pressure is a major determinant of large artery remodelling. Clin Exp Pharmacol

Physiol 2001:28:1011-1014.
28. Tada S, Dong C, Tarbell JM. Effect of the stress phase angle on the strain energy density of the endothelial plasma membrane. Biophys J. 2007:93:3026-3033.
29. Kizer JR, Dahlof B, Kjeldsen SE et.al. Stroke reduction in hypertensive adults with cardiac hypertrophy randomized to losartan versus atenolol: the Losartan Intervention For Endpoint reduction in hypertension study. Hypertension. 2005:45, 46-52.
30. Schrader J, Luders S, Kulschewski A, Hammersen F, Plate K, Berger J, Zidek W, Dominiak P, Diener HC. Morbidity and Mortality After Stroke, Eprosartan Compared with Nitrendipine for Secondary Prevention: principal results of a prospective randomized controlled study (MOSES). Stroke 2005:36:1218-1226.
31. Schrader J, Kulschewski A, Dendorfer A. Inhibition of the renin-angiotensin system and the prevention of stroke. Am J Cardiovasc Drugs. 2007:7, 25-37.
32. Yusuf S, Diener HC, Sacco RL et.al. Telmisartan to prevent recurrent stroke and cardiovascular events. N Engl J Med 2008:359:1225-1237.

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