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KININS MEDIATE THE ANTIPROLIFERATIVE EFFECT OF RAMIPRIL IN RAT CAROTID ARTERY

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Angiotensin-converting enzyme (ACE) inhibitors have been shown to inhibit neointimal proliferation in response to endothelial injury in the rat carotid artery. Since ACE inhibitors block degradation of kinins, our objective in this study was to determine whether kinins mediate the antiproliferative effect of the ACE inhibitor ramipril. Endothelial denudation was achieved in the left carotid artery of male Sprague-Dawley rats using a balloon catheter. The rats were divided into four groups: a) vehicle (saline); b) DuP 753 10 mg/kg/day; c) ramipril 5 mg/kg/day; and d) ramipril 5 mg/kg/day plus Hoe 140 70 μg/kg/day. Ramipril markedly reduced neointimal proliferation compared to control (vehicle) ($p < 0.05$) and DuP 753-treated groups ($p < 0.05$) . When ramipril was given together with Hoe 140 its effect was significantly blunted ($p < 0.05$). These results show that kinins are important mediators in the antiproliferative effect of ACE inhibitors.

Abnormal neointimal hyperplasia with extensive smooth muscle cell (SMC) proliferation is the main feature of the atherosclerotic process which occurs after endothelial denudation (1). Soon after injury, quiescent SMCs are activated and begin to proliferate in the media; they then migrate across the internal elastic lamina into the intima, where proliferation continues, reaching its maximum 2 weeks after injury (2). All this process will finally lead to luminal estenosis. The intrinsic mechanisms that produce activation and migration of SMCs are not fully understood, and several mitogens have been implicated in this process (3). Angiotensin II (Ang II), a potent vasoconstrictor, plays a controversial role in SMC proliferation *in vitro*. When it is added to SMCs cultured in free-serum medium, it

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The abbreviations used are: SMC, smooth muscle cells; ACEi, angiotensin converting enzyme inhibitors; Ang II, angiotensin II; EDRF/NO, endothelium derived relaxing factor/nitric oxide; SHR, spontaneous hypertensive rats.

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increases protein synthesis but not the number of cells (4,5); however when it is added in the presence of serum-containing medium on SMCs from SHR, the number of cells increases suggesting that Ang II may act as a mitogenic co-factor for SMC proliferation (6).

Powell et al. have shown that angiotensin-converting enzyme inhibitors (ACEi) reduce neointimal proliferation after endothelial injury in the rat carotid artery (7,8), suggesting that Ang II plays a major role in this process. However, one of the effects of ACEi is the reduction in the breakdown of endogenous kinins. Kinins can release prostanoids and other factors from endothelial and/or blood cells such as EDRF/NO, which may have an antiproliferative effect (9,10,11) and therefore may also mediate part of the protective effect of ACEi. Our objective in this study was to determine whether kinins do indeed mediate part of this antiproliferative effect.

MATERIAL AND METHODS

Arterial Injury Model

Endothelial denudation was produced in the left common carotid artery of male Sprague-Dawley rats (450-500 g). The rats were anesthetized with sodium pentobarbital 50 mg/kg i.p. and the left common and external carotid arteries were exposed. A balloon catheter was passed through the left external carotid artery and advanced to the aorta; the balloon was then inflated with saline to slightly distend the common carotid and resist withdrawal, and pulled back to the external carotid. This procedure was repeated three times, after which the catheter was removed, the external carotid ligated and the incision sutured(12).

Morphology

Fourteen days after surgery, the rats were anesthetized; a catheter was placed in the thoracic aorta *via* the left femoral artery and the vascular system was first rinsed with phosphate-buffered saline (PBS), then perfused-fixed with 2.5% glutaraldehyde using a perfusion pressure of 100 mm Hg. Both carotid arteries were removed, cleaned of connective tissue, placed in 2.5% glutaraldehyde for further fixation and embedded in paraffin. The arteries were divided into three equal segments going from the proximal to the distal end: lower (closest to the aortic arc), middle, and upper (the last third, near the bifurcation). Several cross-sectional rings (1 μm) were cut from each segment and stained with hematoxylin-eosin. The slices were projected with a Prado-Leitz projecting microscope at a magnification of x100. The lumen, neointima and media were traced on paper and these areas measured using a Sigma-Scan (Jandel Scientific) morphometric computer system. Since no differences were found between the 3 segments their areas were averaged. The results were expressed as a percentage of the ratio neointimal area ÷ medial area.

The rats were divided into four groups. Group A was given vehicle (saline). Group B received the Ang II receptor antagonist DuP 753 (10 mg/kg/day). Group C was given ramipril (5 mg/kg/day), and group D received both ramipril (5 mg/kg/day) and the kinin receptor antagonist Hoe 140 (70 μg/kg/day). All drugs were dissolved in saline and given intraperitoneally as a continuous infusion using an osmotic pump (Alzet 2ML2, Alza, CA). The pumps were implanted in the peritoneal cavity under ether anesthesia 2 days before endothelial denudation.

On day 14 after de-endothelialization, the animals were anesthetized with sodium pentobarbital 50 mg/kg i.p. and mean blood pressure was measured using a femoral catheter connected to a transducer. To assess the effect of the drugs and the degree of blockade of

the various receptors, they were challenged with 100 ng i.v. of Ang I, Ang II and bradykinin (Bk) and the delta blood pressure monitored. All rats received food and water *ad libitum*. Systolic blood pressure was measured twice a week by the tail cuff method. The statistical analysis was performed using analysis of variance, and Tukey's studentized range test was used to adjust for multiple pairwise comparisons. The 0.05 significance level was used.

RESULTS

There was no difference in body weight between the different groups before surgery and at the end of 2 weeks. Systolic blood pressure dropped significantly in the groups treated with DuP 753, ramipril and ramipril plus Hoe 140, compared with vehicle, but no differences were found in the three treated groups.

On day 14 after balloon denudation, neointimal proliferation was significantly lower ($p < 0.05$) in the groups treated with DuP 753 and ramipril. Both drugs were given in doses that produce the same drop in systolic blood pressure [130.4 ± 3.2 to 111.8 ± 1.8 mm Hg for DuP 753 ($n = 10$), and 130.3 ± 2.0 to 111.4 ± 1.2 mm Hg for ramipril ($n = 11$)]. Moreover, these doses blocked the change in blood pressure produced by equimolar i.v. injections of either Ang I or Ang II by nearly the same degree (79% for DuP 753 and 80% for ramipril).

Ramipril afforded significantly more protection against proliferation than DuP 753 ($p < 0.05$); however, its effect was blunted significantly by Hoe 140. The neointima/media ratio was significantly higher in the rats given both ramipril and Hoe 140 than in those given ramipril alone ($p < 0.05$) and was also similar to the ratio seen in the DuP 753-treated rats (p = ns) (table 1 and fig. 1).

No differences in medial area were found between the different treatments; however the luminal area was significantly higher in the ramipril-treated group than in the controls and DuP 753 treated group (table 1).

Table 1. Effects of ramipril, DuP 753 and ramipril + Hoe 140 on balloon catheter-induced vascular injury

Group	n	Lumen Area (μm^2)	Neointima Area (μm^2)	Neointima/Media (%)
Vehicle	10	91.1 ± 10.7	86.6 ± 9.7	107.01 ± 8.25
DuP 753	10	108.7 ± 9.7	53.5 ± 9.4	67.15 ± 10.97*
Ramipril	11	136.6 ± 9.9*†§	17.1 ± 2.4	24.35 ± 4.05*§†
Ramipril + Hoe 140	8	83.0 ± 5.8	49.6 ± 8.3	70.57 ± 10.40*

* $p < 0.05$ compared to vehicle group.
§ $p < 0.05$ compared to DuP 753 group.
† $p < 0.05$ compared to ramipril + Hoe 140 group.

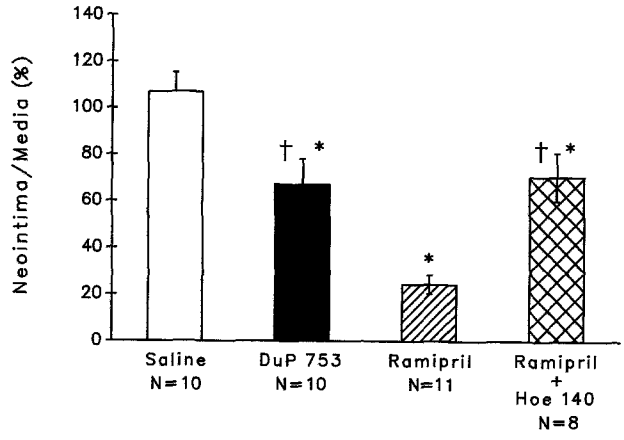

Figure 1.

Changes in neointima formation 2 weeks after carotid artery balloon de-endothelization in rats chronically infused with vehicle (saline), DuP 753 10 mg/kg/day, ramipril 5 mg/kg/day, and ramipril plus Hoe 140 70 μg/kg/day. (*) $p < 0.05$ compared with vehicle, (†) $p < 0.05$ compared with ramipril.

DISCUSSION

It has been demonstrated that ACEi prevent morphological alterations in vessels subjected to endothelial injury (7). It has generally been assumed that most if not all of the effects derived from chronic treatment with ACEi are due to blockade of angiotensin II formation (8,13). However, ACEi can decrease degradation of kinins, since ACE is one of the more important kininases (14). Thus we and others have suggested that some of the effects of ACEi are mediated by kinins (15).

The data we present here provide, for the first time, strong evidence that endogenous kinins mediate part of the antiproliferative effect of ramipril, a potent ACEi, since administration of the kinin receptor antagonist reversed part but not all of the antiproliferative effect of ramipril. DuP 753 also exhibited a statistically significant antiproliferative effect, but ramipril alone was more effective. There was no difference in neointima formation between rats treated with DuP 753 and the group treated with ramipril and Hoe 140. Thus the kinin antagonist eliminated the diffence between ramipril and DuP 753, suggesting that ramipril acts by both inhibiting Ang II formation and increasing endogenous kinins.

There is little information to clarify how increases in endogenous kinins help block vascular proliferative responses, since kinins themselves are thought to be weakly mitogenic (16). One possibility is that kinins act indirectly by increasing the release of endothelium-derived factors such as EDRF/NO and PGI_2, which may be antiproliferative (9,11). Kinins

are known to induce release of these substances from endothelial cells (17). Dzau et al. have hypothesized that the endothelium regulates the proliferative response; however they believe that de-endothelialization removes an inhibitory mechanism, allowing unfettered activity of Ang II (13). While the present results do not contradict this hypothesis, they suggest that kinins may initiate this putative endothelial mediation. However, since no endothelial cells are present, at least during the first week (unpublished observations,18), there may be an alternative source of EDRF/NO or antiproliferative compounds of a similar nature. One possibility is that white blood cells such as monocytes are the source of the antiproliferative mediators stimulated by endogenous kinins. It has previously been shown that kinins stimulate the release of PGE_2 from monocytes (19) and that macrophages can also produce EDRF (18). Another possibility is that kinins accelerate the process of re-endothelialization.

Another question raised by the present results is the origin of the endogenous kinins involved in the response to ACEi. Since we have shown that there is no significant increase in plasma kinin levels after ACE inhibition (20), one possibility is that they originate in the arterial wall. Our laboratory has shown that blood vessels contain both kallikrein activity and mRNA for glandular kallikrein, suggesting that the vascular wall synthesizes kallikrein (21,22).

In summary, we have demonstrated that chronic blockade of kinins results in partial suppression of the antiproliferative effect of ramipril, an ACEi. These results suggest that part of the protective effect of ACEi is due to increases in endogenous kinins.

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REFERENCES

1. Ross R, and Glomset, J.A. (1976) N. Engl. J. Med. 295, 369-377, 1976.

2. Clowes, A.W., Reidy, M.A., and Clowes, M.M. (1983) Lab. Invest. 49, 327-333.

3. Ferns, G.A.A., Raines, E.W., Sprugel, K.H., Motani, A.S., Reidy, M.A., and Ross, R. (1991) Science 253, 1129-1132.

4. Geisterfer, A.A.T., Peach, M.J., and Owens, G.K. (1988) Circ. Res. 62, 749-756.

5. Berk B.C., Vekshtein, V., Gordon, H., and Tsuda, T. (1989) Hypertension 13, 305-314.

6. Burden T.S., Resnik, T.J., and Bühler, F.R. (1991) Am. J. Hypertens. (in press).

7. Powell, J.S., Clozel, J.P., Müller, R.K.M., Kuhn, H., Hefti, F., Hosang, M., and Baumgartner, H.R. (1989) Science 245, 186-188.

8. Powell, J.S., Müller, R.K.M., Rouge, M., Kuhn, H., Hefti, F., and Baumgartner, H.R. (1990) J. Cardiovasc. Pharmacol. 16 (suppl 4), s42-s49.

9. O'Connor, K.J., Knowles, R.G., and Patel, K.D. (1991) J. Cardiovasc. Pharmacol. 17 (suppl. 3), s100-s103.

10. Garg, U.C., and Hassid, A. (1989) J. Clin. Invest. 83, 1774-1777.

11. Loesberg, C., van Wijk, R., Zandbergen, J., van Aken, W.G., van Mourik, J.A., and de Groot, P.G. (1985) Exp. Cell. Res. 160, 117-125.

12. Baumgartner, H.R. (1963) Z. Ges. Exp. Med. 137, 227.

13. Dzau, V.J., Gibbons, G. and Pratt, R.E. (1991) Hypertension 18 (suppl II), II-100-II-105.

14. Erdös, E.G. and Skidgel, R.A. (1987) Lab. Invest. 56, 345-348.

15. Carretero, O.A. and Scicli, A.G. (1991) Hypertension 68, 366-371.

16. Dixon B.S., Breckon, R., Fortune, J., Vavrek, R.J., Stewart, J.M., Marzec-Calvert, R., and Linas, S.L. (1990) Am. J. Physiol. 258, C299-C308.

17. Moncada, S., Palmer, R.M.J., and Higgs, E.A. (1991) Pharmacol. Rev. 43, 109-142.

18. Haudenschild, C.C., and Schwartz, S.M. (1979) Lab. Invest. 41, 407-418.

19. Lerner, U.H., Sahlberg, K., and Ljuggren, Ö. (1989) J. Oral Pathol. Med. 18, 246-250.

20. Carbonell, L.F., Carretero, O.A., Stewart, J.M., and Scicli, A.G. (1988) Hypertension 11, 239-243.

21. Nolly, H., Scicli, A.G., Scicli, G., and Carretero, O.A. (1985) Circ. Res. 56, 816-621.

22. Saed, G.M., Carretero, O.A., MacDonald, R.J., and Scicli, A.G. (1990) Circ. Res. 67, 510-516.