SMH ST. MICHAEL'S HOSPITAL Cardiology UNIVERSITY OF TORONTO

A REPORT BY THE DIVISION OF CARDIOLOGY
ST. MICHAEL'S HOSPITAL, UNIVERSITY OF TORONTO

Scientific Update™

Is ACE inhibition the standard of care for management of CAD? Tissue mechanisms to impact clinical outcomes

Originally presented by: Carl J. Pepine, MD, Helmut Drexler, MD, Kenneth E. Bernstein, MD, Eva Lonn, MD, John Deanfield, MD, Harold L. Lazar, MD.

A Symposium presented at the 72nd Scientific Sessions of the American Heart Association

November 7-10, 1999 Atlanta, Georgia

Reported and discussed by:
JUAN CARLOS MONGE, MD

Few therapeutic interventions have been as extensively investigated in well-designed clinical trials as the use of inhibitors of the angiotensin-converting enzyme (ACE). This *Cardiology Scientific Update* will present an overview of trials from the last few years that have studied the effects of ACE inhibition in patients with heart failure, left ventricular dysfunction, myocardial infarction, angina, and in patients at high risk of ischemic events. It is important to note that ACEIs with different tissue-ACE binding and inhibition properties may produce different functional and structural outcomes despite equivalent inhibition of the circulating ACE. Studies in gene knockout mice have demonstrated that tissue ACE is the most important component of ACE activity and that its absence cannot be compensated by normal levels of circulating ACE in terms of blood pressure effects. An overview of results from the HOPE trial reveals that use of the high-tissue affinity ACEI ramipril resulted in significant reductions in the risk of death, MI, stroke, heart failure-related endpoints, and revascularization procedures. It would appear that high tissue-affinity ACE inhibition, by improving nitric oxide bioavailability and correcting endothelial dysfunction, exerts potent anti-atherogenic effects thus preventing cardiovascular events beyond the benefits of current therapeutic strategies such as lipid lowering.

A review of ACE inhibitor trials

In the treatment of heart failure, the first major clinical outcome trial was the CONSENSUS study in the 1980s. This Scandinavian trial concentrated principally on NYHA class IV patients, the majority on the basis of ischemic heart disease. CONSENSUS was stopped prematurely because of the marked benefit seen with the ACEI enalapril.[1]

While CONSENSUS was in progress, the first large North American trial was initiated, also with enalapril. SOLVD included patients who had functional class II and III heart failure and demonstrated the survival benefits of ACE inhibition on less symptomatic patients.[2] Importantly, SOLVD also included an arm – the Prevention Trial – that included approximately 4,500 asymptomatic patients with left ventricular dysfunction, primarily as a result of a remote myocardial infarction (MI). The Prevention Trial did not achieve a statistically significant benefit for survival, but it demonstrated for the first time that ACE inhibition could retard the progression of heart failure.[3]

The results of these earlier studies, in terms of heart failure benefits, were not surprising given the available experimental data showing that heart failure was associated with activation of the renin-angiotensin system (RAS) and that administration of an ACEI altered the hemodynamic state and improved heart failure symptoms. What was unexpected in these early studies was the significant decrease observed in adverse outcomes related to ischemic heart disease such as MI and unstable angina.[4]

These striking results were subsequently confirmed in the SAVE study, a trial of late initiation of ACEI with captopril in post-MI patients with decreased ejection fraction.[5,6] Further demonstration of the consistency of the benefits of ACEI in MI survivors with left ventricular dysfunction was provided by several other trials: TRACE (trandolapril), SMILE (lisinopril), and AIRE (ramipril).[7-9] Remarkably, these five trials demonstrated a

St. Michael's Hospital, 30 Bond St., Suite 9-004, Queen Wing, Toronto, Ontario M5B 1W8 Fax: (416) 864-5330

The topics presented in *Cardiology Scientific Update* are independently determined and the content authored exclusively by physician-members of the Division of Cardiology, St. Michael's Hospital. *Cardiology Scientific Update* is made possible by unrestricted funding from the publisher, Snell Medical Communication Inc., which has received educational grants from the pharmaceutical industry for the distribution of this publication.

consistent reduction in the incidence of recurrent MI of 7-8% per year of treatment.

Additional clinical studies have demonstrated that the benefits of ACE inhibition extend to patients treated early, within 24 to 36 hours of presentation, independently of left ventricular function. These findings suggested that patients with ischemic heart disease who do not have heart failure or severe left ventricular dysfunction would benefit from this intervention. Indeed, this benefit has been estimated at 5 lives saved per 1,000 patients treated and emerged very early in the course of treatment (within the first three days).[10] The combined results of this large body of clinical trials is consistent with the possibility that the entire post-MI population, from low risk asymptomatic patients to NYHA functional class IV patients, stand to benefit from this remarkable therapeutic intervention.

Effects of ACEI on surrogate outcomes in patients with CAD

A number of trials that included patients with coronary artery disease (CAD) have examined the effects of ACEI on surrogate outcomes. Some of these studies were well designed, randomized, clinical trials that supported the direction of the findings of the morbidity and mortality trials and provided useful information about potential mechanisms.

The effects of ramipril have been studied in multiple clinical trials and this is of great interest as this is a potent ACEI with a long half-life and a high tissue-affinity which would be expected to be more effective at inhibiting the tissue renin-angiotensin system (RAS) than older agents with lower tissue affinity. A recently published study examined the effects of ramipril or placebo in a randomized, parallel, double-blind trial in patients with chronic stable CAD and preserved systolic left ventricular function. After six months of treatment, ramipril improved resting left ventricular function and both resting and exercise-induced diastolic filling abnormalities suggesting, that tissue ACE inhibition was beneficial in myocardial ischemia.[11]

The TREND study, published a few years ago, included patients with CAD and coronary endothelial dysfunction defined by an abnormal response to acetylcholine. These patients had normal left ventricular function and no severe hypertension or dyslipidemia. After six months of therapy with quinapril, also a potent ACEI with high tissue affinity, there was a highly significant improvement in coronary endothelial-mediated vascular function.[12]

A more recent study, conducted in Canada, the Brachial Artery Normalization of Forearm Flow (BANFF) trial, assessed the effects of quinapril, enalapril, losartan, and amlodipine in doses selected to produce equivalent blood pressure reduction in patients with chronic stable CAD. In BANFF, endothelial function was evaluated noninvasively by ultrasound determination of flow-mediated dilatation of the brachial artery. Treatment with quinapril was the only intervention that significantly improved endothelial function, consistent with the hypothesis that in patients with CAD, the use of an ACEI with high tissue affinity is superior in this regard to other blood pressure lowering interventions, including an ACEI with lower tissue affinity.[13]

Additional evidence to support the beneficial effects of ACEI in CAD patients was provided by a recent European study named QUO VADIS (Effects of Quinapril On Vascular ACE and Determinants of Ischemia).[14] In this study, 149 patients scheduled to undergo elective coronary bypass surgery were randomized to quinapril, captopril – an ACEI with lower tissue affinity – or placebo for 7 days prior to their operation. At the time of surgery, a segment of the left internal mammary artery was harvested and its endothelial function was evaluated by conventional methods. Only quinapril improved endothelial function significantly, relative to placebo, despite a similar decline in blood pressure. After the operation, the patients were continued on either 40 mg quinapril or placebo for one year and followed for pre-specified ischemic events. Quinapril treatment of these patients, who did not have evidence of heart failure or severe left ventricular dysfunction, resulted in a remarkable 80% decrease in the incidence of ischemic events compared to placebo (4% vs 18%, $p = 0.03$), suggesting that the benefits of ACEI may extend to yet another important sub-population of patients with ischemic heart disease.

Effects of ACEI on clinical events

Five major outcome trials, concluded or in progress, were designed to evaluate the effects of ACEI on morbidity and mortality in patients with CAD or at risk of CAD.

- The QUIET trial used quinapril in a very low-risk population of 1,750 patients with documented CAD. Although there was a trend toward a reduction in cardiovascular events with quinapril, the results did not achieve statistical significance.[15] In retrospect, QUIET was a study that was likely underpowered to detect a significant difference as the event rate in the placebo arm was rather low, consistent with the low risk profile of the patient population, and thus insufficient to show a significant difference relative to treatment with quinapril. An additional factor that could explain the results was that the dose of quinapril utilized in the study (20 mg) would now be generally accepted to be, at best, an intermediate dose. Indeed, in the TREND study the dose of quinapril that was associated with an improvement in endothelial function was 40 mg.
- The recently reported HOPE study, which included 9,514 high-risk patients, used high dose ramipril and demonstrated conclusively the benefits of ACEI on the prevention of cardiovascular events in patients without severe left ventricular dysfunction.[16] HOPE will be discussed in greater detail in a subsequent section of this *Cardiology Scientific Update*.

• The ongoing Antihypertensive and Lipid Lowering Treatment To Prevent Heart Attack Trial (ALLHAT) is evaluating, among other interventions, the effects of lisinopril in hypertensive patients at high risk, but without overt CAD.[17,18]

• In the ongoing Prevention of Events with ACE Inhibition study (PEACE), the effects of the potent ACEI trandolapril are being evaluated in 8,100 patients with CAD and normal left ventricular function.[19]

• The European Trial on Reduction of Cardiac Events with Perindopril in Stable Coronary Artery Disease (EUROPA) is evaluating the effects of ACE inhibition in 10,500 patients with stable CAD and no clinical heart failure.[20]

It is quite obvious therefore, that few, if any, therapeutic interventions have been as extensively evaluated in well-designed clinical trials as the use of ACEIs. With a remarkable consistency, ACEIs have been shown to decrease major cardiovascular outcomes in a broad range of patients with or without heart failure or left ventricular dysfunction and across practically all stages of the risk spectrum.

Mechanisms of ACE inhibition: Are all ACE inhibitors created equal?

Although the remarkable benefit of ACEI in the prevention of ischemic events was fairly consistent in earlier trials such as SOLVD, it is important to note that this was a retrospective analysis that must be interpreted with caution. The HOPE study, on the other hand, is a landmark study not only because it evaluated the benefits of ACEI in a different population (patients without heart failure or left ventricular dysfunction), but it also did so in a prospective fashion with pre-specified ischemic events as major endpoints. In the context of a vast body of epidemiological data, it is unlikely that the entire benefit of ramipril in HOPE can be explained on the basis of blood pressure lowering. It is reasonable to postulate, thus, that ramipril, an inhibitor with high affinity for tissue ACE, exerted direct vasculoprotective effects that go beyond blood pressure reduction.

The biological basis for high-affinity ACE inhibition may be related to the presence of two active catalytic sites on ACE.[21] All ACEI are able to interact with the first active site, but the structure of specific ACEI, such as quinapril and ramipril, would predict an increased interaction with the second hydrophobic active site.[22] High-affinity ACEIs have the potential, therefore, to achieve more effective inhibition of the tissue ACE. Several studies have provided experimental support for this concept. Ruzicka and Leenen used a rat model of myocardial hypertrophy to compare the effects of enalapril and quinapril in dosages that were equipotent in their inhibition of the circulating ACE as measured by the pressor response to an angiotensin I infusion. The consequences of long-term treatment with the two agents were, however, quite different. Enalapril resulted in only a slight decrease in left ventricular hypertrophy, whereas treatment with quinapril led to a much more significant reduction.[23] These results are consistent with the concept that ACEI with different tissue-ACE binding and inhibition properties may produce markedly different functional and structural outcomes despite equivalent inhibition of the circulating ACE.

Similarly, studies of endothelial function have demonstrated significant differences between the effects of different ACE inhibitors. A recent study compared the ability of enalapril and quinapril to improve flow-dependent vasodilatation, an index of nitric oxide bio-availability and endothelial health. This study also utilized dosages of both agents that were equivalent in their pressor response to an infusion of angiotensin I. Quinapril produced a significant 40% increase in flow-dependent vasodilatation, whereas no significant effect was seen with enalapril despite a similar inhibition of circulating ACE.[24] Additional studies have established directly that the improvement in vasodilatation seen with quinapril is related to an increase in nitric oxide bioavailability.[25]

If endothelial function is a surrogate marker of vascular protection, the effects seen in these experimental studies would suggest that there may be differences between ACEIs in their ability to reduce cardiovascular events. This is, however, merely a speculative concept at this time as no comparative outcome-based clinical studies have been performed to evaluate this question. Indeed, it was not until the results of HOPE were released a few months ago that the very notion of preventing cardiovascular events with ACEI in normotensive patients with normal left ventricular function was, for the first time, supported by a large, well-designed, multicenter clinical trial.

Tissue ACE in cardiovascular regulation: Lessons from genetic manipulation

Some of the most remarkable insights about the function of the renin-angiotensin system since its discovery more than 100 years ago, have been gained recently from studies using recombinant DNA techniques in intact animals, namely transgenic mice and gene knockout experiments. These experiments are particularly important in defining the functions of ACE in view of its lack of substrate specificity in contrast to renin which is highly substrate-specific.

ACE is formed by a single polypeptide chain with four important functional domains. At the amino-terminal section of the protein there is a signal sequence which allows the protein to be exported from the cells. There are as well two catalytic active sites, named the amino-terminal and the carboxyl-terminal catalytic domains from their location in the protein. Finally, at the carboxyl terminus of the molecule there is a hydrophobic region which mediates its insertion into the cell membrane and prevents the secretion of the protein. Gene knockout experiments allow the targeted and specific deletion of a gene in embryonic

stem cells and the generation, from those pluripotential cells, of whole animals that lack expression of the gene of interest. The functions of the "knocked out" gene can then be studied on the basis of the abnormalities present in the animals.

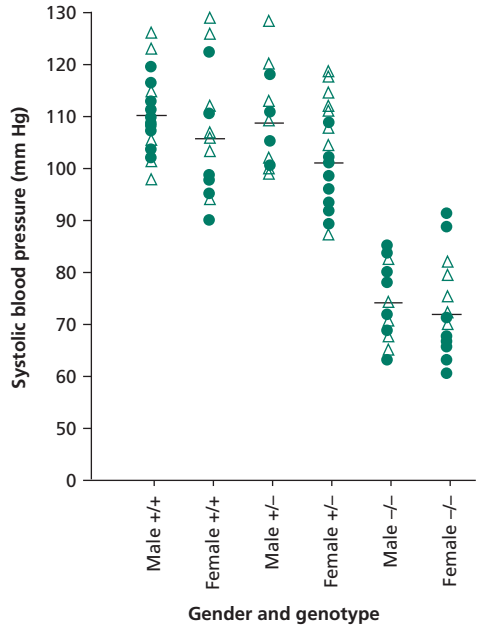
A knockout of the entire ACE gene has been reported which completely lacks the expression of the gene, resulting in animals that do not express tissue or plasma ACE from their conception and constitute an extreme phenotype for the absence of ACE activity (ACE.1 mice, Figure 1). The homozygous knockout mice (–/–) had a mean systolic blood pressure of 73 mm Hg compared to 110 mm Hg in normal animals. This result is of great significance because it means that there is no mechanism to compensate for the absence of ACE despite the complexity of the regulation of blood pressure and the multiple existing vasopressor factors such as catecholamines and endothelin. As well, the ACE knockout mice present severe developmental abnormalities of the renal medulla and papillae and an impaired ability to concentrate urine.

A second knockout animal was developed by the same investigators which maintains the signal sequence and the amino-terminal catalytic domain, but lacks the carboxyl-terminal catalytic domain and the hydrophobic region (ACE.2, Figure 1). This partially truncated gene results in an active ACE that is entirely secreted because of the absence of the domain that allows its normal anchoring to the cell membrane. In other words, these animals represent a gene knockout for tissue ACE but have preserved circulating ACE expression. Interestingly, the tissue-ACE knockout mice also had low blood pressure which was no different from the animals that lacked the expression of the entire ACE gene. As well, the levels of plasma angiotensin II were decreased to the same extent in the full ACE gene knockout and in the tissue ACE knockout. These results indicate that tissue ACE is the most important component of ACE activity as its absence cannot be compensated by normal levels of circulating ACE in terms of blood pressure effects.[26-28]

Tissue ACE inhibitors and their effects on outcomes: Findings from HOPE and HOPE sub-studies

The Heart Outcomes Prevention Evaluation (HOPE) trial included patients who were ≥55 years of age and at high risk for cardiovascular events because of pre-existing vascular disease, or the presence of diabetes and additional risk factors for vascular disease. HOPE included more than 9,500 patients, in a 2 x 2 factorial design, randomized to ramipril 10 mg versus placebo and vitamin E versus placebo. The large number of patients and the long follow-up

Figure 1: The systolic blood pressures of male and female wild-type (+/+), heterozygous (+/–), and knockout (–/–) mice were determined by tail-cuff measurement. Data from ACE-null mice (ACE.1) are represented as triangles (△), while data from the ACE.2 mice are closed circles (●). The ACE.2 knockout mice have blood pressures that are virtually identical to mice lacking all ACE.



were incorporated in the design to give the study the power to detect significant effects, not only on the primary study endpoint (ie, a composite of cardiovascular death, MI and stroke), but also on each component of the primary endpoint individually, as well as other clinically relevant endpoints such as need for revascularization, heart failure, unstable angina and diabetes and its complications. As well, the study allowed the evaluation of multiple pre-defined subgroups. It is important to note that less than half of the enrolled patients were hypertensive and that the patients did not have evidence of overt heart failure or left ventricular dysfunction representing, therefore, a population in which ACE inhibitors have not been considered as being indicated on the basis of the available evidence.[16]

The use of the high-tissue affinity ACEI ramipril in HOPE resulted in a 22% reduction in the composite primary endpoint, a 25% reduction in the risk of death, a 20% reduction in the risk of MI, a 31% reduction in the risk of

stroke, a 22% reduction in heart failure-related endpoints and 16% reduction in revascularization procedures. Interestingly, and in concordance with data from the CAPPP trial which evaluated the effects of ACEI in hypertension,[29] there was a significant 31% reduction in new cases of diabetes mellitus. Additionally, both macrovascular complications of diabetes and diabetic nephropathy were also significantly reduced. These extraordinary benefits came at a relatively small cost as there was only a 5% excess of cough in the patients taking ramipril, whereas other adverse effects were not statistically different from placebo.

An extremely important issue in HOPE is whether the modest reduction in blood pressure achieved with ramipril treatment (3.3 mm Hg decrease in mean systolic pressure) is sufficient to explain the remarkable benefits observed. It must be emphasized that HOPE was not an antihypertensive trial and hypertension was not, therefore, the primary reason for enrollment. As a concurrent condition, hypertension was present in less than 50% of the patients and most were receiving concomitant therapy. Indeed, >40% of the patients were treated with ß-blockers, >40% were on calcium channel blockers, and a total of 75% were on antiischemic therapy in general. The baseline mean systolic blood pressure on entry was 139 mm Hg and the mean diastolic blood pressure was 78 mm Hg, hardly the values of a typical hypertensive cohort. These reasons likely account for the small blood pressure reduction seen in the study. Extrapolation from data available in the hypertension literature predicts that such blood pressure reduction would result in only a 13% reduction in the risk of stroke and a 5% reduction in the risk of MI.

Without ignoring the limitations of this approach, it is reassuring that the HOPE study actually found that the reductions in the risk of stroke and MI were more than twice and more than four-fold, respectively, what would be expected from blood pressure lowering alone. It is likely that ramipril, an ACEI with high tissue affinity, exerted direct vasculoprotective effects beyond those derived from a reduction in blood pressure. This concept is supported as well by post-hoc analysis of the HOPE data showing that the reduction in clinical events was observed in all quartiles of baseline and on-treatment systolic and diastolic blood pressure.

The direct vasculoprotective effects of ACEI have been extensively documented in experimental animal models. For instance, over a decade ago, Chobanian and co-workers demonstrated the anti-atherogenic effects of ACEI in a hyperlipidemic animal model.[30,31] Similar data have been reported in non-human primates, such as the cynomolgus monkey, on an atherogenic diet.[32] Additional animal studies have demonstrated that a high cholesterol diet is associated with a marked induction in aortic ACE activity which co-localizes with the development of atherosclerotic lesions. The administration of a high tissue-affinity ACEI, resulted in a reduction of aortic tissue-ACE levels which correlated with a marked suppression of lesion development.[33,34]

The HOPE study evaluated the evidence of direct vasculoprotective effects in a substudy called SECURE.[35] This substudy included 732 patients who were evaluated by B-mode carotid ultrasound at baseline and with serial studies during the duration of the HOPE trial. The technique of B-mode carotid ultrasound allows direct measurement of the combined intimal and medial thickness of the vessel, a parameter that has been extensively validated, not only as representative of the atherosclerotic involvement of the vessel by histological measurements, but also as a potent predictor of cardiovascular events and the concomitant presence of coronary artery disease. In the SECURE study, treatment with ramipril 10 mg daily resulted in a significant 37% lower progression rate in the serial measurements of the combined intimal-medial thickness. These results provide direct evidence that ACEI delayed progression of the atherosclerotic process in this subgroup of patients of the HOPE study, and illustrate one of the possible mechanisms to explain the clinical benefits of ACE inhibition beyond blood pressure lowering.

Conclusion

Evidence is mounting to support the endothelial function hypothesis of atherosclerosis which postulates that dysfunction of the endothelium is at the root of the development of this disease. High tissue-affinity ACEI, by improving nitric oxide bioavailability and correcting endothelial dysfunction, would exert potent anti-atherogenic effects resulting in the prevention of cardiovascular events beyond the benefits of current therapeutic strategies such as lipid lowering. The acceptance of this hypothesis would lead to an extension of the benefits of ACEI to large populations of patients who are currently not necessarily treated with these agents, such as normotensive patients with vascular disease and normal left ventricular function. The recently reported HOPE trial provides strong evidence to support the use of ACEI in those patient populations, as well as their use in the primary prevention of cardiovascular events in high-risk diabetic patients. The results of ongoing large multicenter trials such as ALLHAT, EUROPA, and PEACE will provide additional information to assess the role of ACE inhibition in the prevention of cardiovascular events. In the meantime, an increased use of these agents in select, but potentially large, additional patient populations is clearly warranted by the remarkable results of the HOPE trial. It would be reasonable

to use high tissue-affinity ACEI for these extended indications on the basis of abundant experimental data although evidence from direct comparative clinical trials is not available.

References

1. Effects of enalapril on mortality in severe congestive heart failure. Results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). The CONSENSUS Trial Study Group. *N Engl J Med* 1987;316(23):1429-1435.
2. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. The SOLVD Investigators. *N Engl J Med* 1991;25(5):293-302.
3. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD Investigators [published erratum appears in *N Engl J Med* 1992 Dec 10;327(24):1768]; *N Engl J Med* 1992;327 (10):685-691.
4. Yusuf S, Pepine CJ, Garces C, et al. Effect of enalapril on myocardial infarction and unstable angina in patients with low ejection fractions. *Lancet* 1992;340(8829):1173-1178.
5. Pfeffer MA, Braunwald E, Moyé LA, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. *N Engl J Med* 1992;327(10):669-677.
6. Rutherford JD, Pfeffer MA, Moyé LA, et al. Effects of captopril on ischemic events after myocardial infarction. Results of the Survival and Ventricular Enlargement trial. SAVE Investigators. *Circulation* 1994;90(4): 1731-1738.
7. Kober L, Torp-Pedersen C, Carlsen JE, et al. A clinical trial of the angiotensin-converting-enzyme inhibitor trandolapril in patients with left ventricular dysfunction after myocardial infarction. Trandolapril Cardiac Evaluation (TRACE) Study Group. *N Engl J Med* 1995; 333(25):1670-1676.
8. Borghi C, Bacchelli S, Esposti DD, Bignamini A, Magnani B, Ambrosioni E. Effects of the administration of an angiotensin-converting enzyme inhibitor during the acute phase of myocardial infarction in patients with arterial hypertension. SMILE Study Investigators. Survival of Myocardial Infarction Long-term Evaluation. *Am J Hypertens* 1999; 12(7):665-672.
9. Effect of ramipril on mortality and morbidity of survivors of acute myocardial infarction with clinical evidence of heart failure. The Acute Infarction Ramipril Efficacy (AIRE) Study Investigators. *Lancet* 1993; 342(8875):821-828.
10. Indications for ACE inhibitors in the early treatment of acute myocardial infarction: systematic overview of individual data from 100,000 patients in randomized trials. ACE Inhibitor Myocardial Infarction Collaborative Group. *Circulation* 1998; 97(22):2202-2212.
11. Willenheimer R, Rydberg E, Oberg L, Juul-Moller S, Erhardt L. ACE inhibition with ramipril improves left ventricular function at rest and post exercise in patients with stable ischaemic heart disease and preserved left ventricular systolic function. *Eur Heart J* 1999;20(22):1647-1656.
12. Mancini GB, Henry GC, Macaya C, et al. Angiotensin-converting enzyme inhibition with quinapril improves endothelial vasomotor dysfunction in patients with coronary artery disease. The TREND (Trial on Reversing ENdothelial Dysfunction) Study [published erratum appears in *Circulation* 1996 Sep 15;94(6):1490]. *Circulation* 1996;94(3):258-265.
13. Anderson TJ, Elstein E, Haber H, Charbonneau F. Comparative study of ACE-inhibition, angiotensin II antagonism, and calcium channel blockade on flow-mediated vasodilation in patients with coronary disease (BANFF Study). *J Am Coll Cardiol* 2000;35:60-66.
14. Oosterga M, Voors AA, Veeger NJ, et al. Beneficial effects of quinapril on ischemia in coronary bypass surgery patients – One-year clinical follow-up of the QUO-VADIS Study. *Circulation* 1999;98(17):I636.
15. Cashin-Hemphill L, Holmvang G, Chan RC, Pitt B, Dinsmore RE, Lees RS. Angiotensin-converting enzyme inhibition as antiatherosclerotic therapy: no answer yet. QUIET Investigators. QUinapril Ischemic Event Trial. *Am J Cardiol* 1999;83(1):43-47.
16. The Heart Outcomes Prevention Evaluation Study Investigators. Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *N Engl J Med* 2000;342(3):145.
17. Elliott WJ. ALLHAT: the largest and most important clinical trial in hypertension ever done in the USA. Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial. *Am J Hypertens* 1996;9(4 Pt 1):409-411.
18. Davis BR, Cutler JA, Gordon DJ, et al. Rationale and design for the Antihypertensive and Lipid Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). ALLHAT Research Group. *Am J Hypertens* 1996; 9(4 Pt 1):342-360.
19. Pfeffer MA, Domanski M, Rosenberg Y, et al. Prevention of events with angiotensin-converting enzyme inhibition (the PEACE study design). Prevention of Events with Angiotensin-Converting Enzyme Inhibition. *Am J Cardiol* 1998; 82(3A):25H-25H.
20. Fox KM, Henderson JR, Bertrand ME, Ferrari R, Remme WJ, Simoons ML. The European trial on reduction of cardiac events with perindopril in stable coronary artery disease (EUROPA). Eur Heart J 1998;19 Suppl J:J52-55
21. Wei L, Alhenc-Gelas F, Corvol P, Clauser E. The two homologous domains of human angiotensin I-converting enzyme are both catalytically active. *J Biol Chem* 1991; 266(14):9002-9008.
22. Deddish PA, Wang LX, Jackman HL, et al. Single-domain angiotensin I converting enzyme (kininase II): characterization and properties. *J Pharmacol Exp Ther* 1996; 279(3):1582-1589.
23. Ruzicka M, Leenen FH. Relevance of blockade of cardiac and circulatory angiotensin-converting enzyme for the prevention of volume overload-induced cardiac hypertrophy. *Circulation* 1995;91(1):16-19.
24. Lyons D, Webster J, Benjamin N. Effect of enalapril and quinapril on forearm vascular ACE in man. *Eur J Clin Pharmacol* 1997;51(5):373-378.
25. Koh KK, Bui MN, Hathaway L, et al. Mechanism by which quinapril improves vascular function in coronary artery disease. *Am J Cardiol* 1999;83(3):327-331.
26. Esther CR, Marino EM, Howard TE, et al. The critical role of tissue angiotensin-converting enzyme as revealed by gene targeting in mice. *J Clin Invest* 1997;99(10):2375-2385.
27. Esther CRJ, Howard TE, Marino EM, Goddard JM, Capecchi MR, Bernstein KE. Mice lacking angiotensin-converting enzyme have low blood pressure, renal pathology, and reduced male fertility. *Lab Invest* 1996; 74(5):953-965.
28. Bernstein KE. The role of tissue angiotensin-converting enzyme (ACE): studies of ACE mutant mice. *Am J Cardiol* 1998;82(10A):5S-5S.
29. Hansson L, Lindholm LH, Niskanen L, et al. Effect of angiotensin-converting-enzyme inhibition compared with conventional therapy on cardiovascular morbidity and mortality in hypertension: the Captopril Prevention Project (CAPPP) randomised trial. *Lancet* 1999;353(9153): 611-616.
30. Chobanian AV, Haudenschild CC, Nickerson C, Hope S. Trandolapril inhibits atherosclerosis in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* 1992;20(4):473-477.
31. Chobanian AV, Haudenschild CC, Nickerson C, Drago R. Antiatherogenic effect of captopril in the Watanabe heritable hyperlipidemic rabbit. *Hypertension* 1990;15(3):327-331.
32. Miyazaki M, Takai S. Antiatherosclerotic effect of alacepril, an angiotensin-converting enzyme inhibitor, in monkeys fed a high-cholesterol diet. *Hypertens Res* 1999;22(1):49-54.
33. Mitani H, Bandoh T, Kimura M, Totsuka T, Hayashi S. Increased activity of vascular ACE related to atherosclerotic lesions in hyperlipidemic rabbits. *Am J Physiol* 1996;271(3 Pt 2):H1065-H1071.
34. Charpiot P, Rolland PH, Friggi A, et al. ACE inhibition with perindopril and atherogenesis-induced structural and functional changes in minipig arteries. *Arterioscler Thromb* 1993;13(8):1125-1138.
35. Lonn EM, Yusuf S, Doris CI, et al. Study design and baseline characteristics of the study to evaluate carotid ultrasound changes in patients treated with ramipril and vitamin E: SECURE. *Am J Cardiol* 1996;78(8): 914-919.