PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 185, 262–266 (1987)

Additive Beneficial Effects of Two Inhibitors of Vasoconstrictor Mediators in Acute Myocardial Ischemia[1] (42542)

HAIM BITTERMAN,[2] DAVID J. LEFER, AND ALLAN M. LEFER

Department of Physiology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, Pennsylvania 19107

Abstract. A specific inhibitor of thromboxane A_2 (TxA_2) synthesis, CGS-12970, a new angiotensin-converting enzyme (ACE) inhibitor, CGS-16617, and a combination of both agents were evaluated for their ability to reduce the extension of myocardial infarct size in rats. Myocardial creatine kinase (CK) loss from the left ventricular free wall (LVFW) 48 hr after left coronary artery ligation was used as an index of ischemic damage. Treatment with either CGS-12970 (4 mg/kg) or CGS-16617 (1 μg/kg) alone did not attenuate the loss of CK from LVFW significantly, compared with animals receiving only the vehicles for these drugs. However, the combined use of both agents significantly reduced CK depletion from LFVW ($P < 0.01$). These findings support the interrelated role of TxA_2 and angiotensin II as mediators of myocardial ischemia and suggest that combined inhibition of their formation may be useful in the treatment of acute myocardial infarction.

The renin–angiotensin system is activated following a variety of perturbations in circulatory function including acute coronary artery ligation (1). Since angiotensin II is a potent systemic and coronary vasoconstrictor and a positive inotropic agent (2), activation of the renin–angiotensin system may increase myocardial oxygen demand, decrease myocardial oxygen supply, and thus contribute to the evolution of myocardial ischemic damage. Angiotensin-converting enzyme (ACE) inhibitors have been shown to exert beneficial effects in several studies of acute myocardial ischemia (3–7). Thus, ACE inhibitors were reported to limit infarct size (3, 4), reduce reperfusion arrhythmias (5, 6), and increase long-term survival during experimental myocardial infarction (7). These beneficial effects have been attributed to a variety of effects including an increase in collateral blood flow to the ischemic region (4), reduced myocardial oxygen consumption (4), and interference with norepinephrine release from presynaptic sympathetic nerve endings independent of angiotensin II inhibition (5).

Thromboxane A_2 (TxA_2), a major eicosanoid produced by the cyclooxygenase pathway of arachidonic acid, is a very potent coronary vasoconstrictor, an inducer of platelet aggregation, and a cytolytic agent (8, 9). Moreover, TxA_2 production increases significantly during acute myocardial ischemia (10, 11). Coronary vasospasm, platelet aggregation, and loss of myocardial cell integrity induced by increased localized production of TxA_2 could propagate ischemic damage once myocardial ischemia is initiated. In this regard, thromboxane synthetase inhibitors and thromboxane receptor antagonists have been reported to reduce the ischemic damage to the myocardium after acute coronary artery ligation (12–14).

The purpose of the present study is to evaluate the combined effect of two new agents, CGS-16617 (3[5-amino-1-carboxy-1s-pentyl]amino-2,3,4,5-tetrahydro-2-oxo-3S-1H-1-bensazepine-1-acetic acid), a selective ACE inhibitor (15), and CGS-12970 (3-methyl-2-[3-pyridyl]-1-indoleoctanoic acid), a TxA_2 synthetase inhibitor, in a 48-hr model of myocardial infarction in rats.

Materials and Methods. Male Sprague–Dawley rats (240–270 g) were anesthetized with ether prior to surgery. Myocardial infarction (MI) was induced using a technique based on that of Selye *et al.* (16) with previously published modifications (3). MI was

[1] Supported by Research Grant HL-25575 of the National Heart, Lung and Blood Institute of the NIH.

[2] Postdoctoral Fellow of the Ischemia-Shock Research Institute of Thomas Jefferson University.

0037-9727/87 $1.50

produced by briefly externalizing the heart from the thoracic cavity through a left thoracotomy and ligating a 4-O silk suture around the left coronary artery 2 to 3 mm from its origin. The heart was repositioned in the thoracic cavity, air was evacuated from the thorax, and the chest wall was rapidly closed by a previously placed purse-string suture. Sham-operated control rats (sham MI) underwent all the same surgical procedures except that the 4-O silk suture, which was passed under the left coronary artery, was not tied. The entire surgical procedure required 1 to 2 min and the rats regained consciousness 2 to 4 min after coronary artery ligation. In this study, 40 rats were subjected to coronary artery ligation. Three of these rats died over the 48-hr observation period and 3 failed to develop an infarct. Thus, the success rate was 85%.

In a previous study (12), the thromboxane synthetase inhibitor CGS-12970, given at a dose of 8 mg/kg, limited ischemic damage following acute coronary artery occlusion in rats. When given at a dose of 4 mg/kg, CGS-12970 did not significantly attenuate creatine kinase (CK) loss from the ischemic tissue. In another study (17), CGS-16617 (10 μg/kg) exerted beneficial effects on hemorrhagic shock in rats. However, when given alone at a lower dose (1 μg/kg) the effects were not significant. In view of these effects of each drug alone, we tested CGS-12970 at a dose of 4 mg/kg and CGS-16617 at a dose of 1 μg/kg.

Rats were divided into the following groups: (i) sham MI + CGS-12970 (4 mg/kg, $n = 6$), (ii) sham MI + CGS-16617 (1 μg/kg, $n = 7$), (iii) sham MI + combined treatment (CGS-12970, 4 mg/kg + CGS-16617, 1 μg/kg, $n = 5$), (iv) MI + vehicle (70% ethanol, $n = 10$), (v) MI + CGS-12970 (4 mg/kg, $n = 7$), (vi) MI + CGS-16617 (1 μg/kg, $n = 7$), and (vii) MI + combined treatment ($n = 10$). Half of the total dose of each drug or their combination was administered over 1 to 2 min (iv bolus) 10 min after coronary ligation and repeated intraperitoneally 4 hr later. Rats given a vehicle received an equal volume of 70% ethanol (0.1 ml) at the same time intervals. Forty-eight hours after coronary artery ligation or sham ligation, the rats were killed by an overdose of ether and the hearts were excised and placed in ice-cold 0.9% NaCl for myocardial tissue analysis.

Tissue analysis. In order to quantify ischemic damage, we measured myocardial CK depletion of the left ventricular free wall (LVFW). This technique has been shown to be a sensitive index of the degree of ischemic damage and correlates well with histologic findings and morphometric measurements of infarct size (18, 19). Atrial and right ventricular free wall tissues were removed and discarded. The interventricular septum was then separated from the LVFW. Both septal and LVFW tissues were homogenized in cold 0.25 *M* sucrose (1:10, w/v) containing 1 m*M* EDTA and 0.1 m*M* mercaptoethanol using a Polytron (Brinkman PCU-2) homogenizer. Homogenates were centrifuged at 36,000*g* at 2°C for 30 min. The supernatants were decanted and assayed for CK activity spectrophotometrically according to the method of Rosalki (20) and for protein concentration by the method of Gornall *et al.* (21). Tissue CK activity was expressed as international units per milligram protein.

Statistical analysis. All values in the text are expressed as mean ± SEM. Student's *t* test for independent means was used for comparison between pairs. Differences among multigroup means were compared by analysis of variance (ANOVA). *P* values of less than 0.05 were considered significant.

Results. In preliminary studies, we found that neither compound at the doses employed exerted any significant effect on arterial blood pressure or heart rate. Figure 1 illustrates the difference in CK activity (Δ myocardial CK) between nonischemic (septum) and ischemic (LVFW) myocardial tissue 48 hr after acute left coronary artery ligation. The three sham MI groups given CGS-12970 or CGS-16617, or the drug combination, exhibited only minimal differences in myocardial CK activity which were not significantly different from zero or from each other. However, acute coronary artery ligation resulted in a significant ($P < 0.001$) depletion of CK from the LVFW of rats given only the ethanol vehicle. The septum-free wall difference (i.e., the Δ CK) myocardial CK values were 1.89 ± 0.14 IU/mg protein. Administration of either CGS-12970 (4 mg/kg) or CGS-16617 (1 μg/kg) alone did not result in a significant attenuation of CK loss from the ischemic tissue. The corresponding myocardial Δ CK values in these

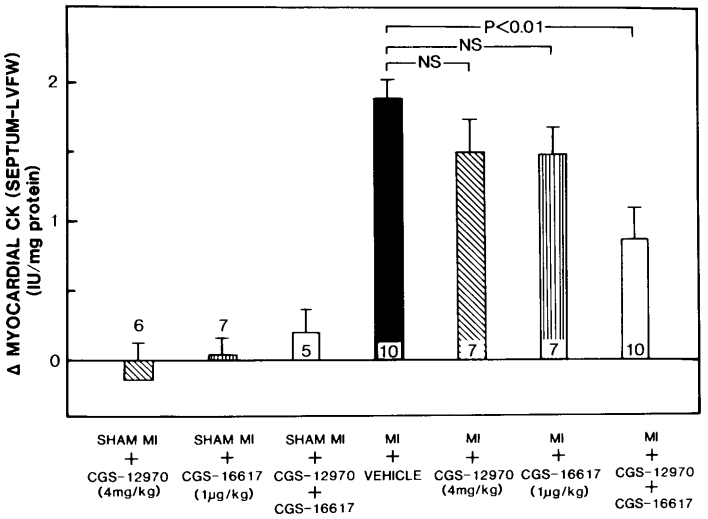

FIG. 1. Myocardial creatine kinase (CK) activities illustrated as the difference between nonischemic (septum) and ischemic (LVFW) myocardium and expressed as international units per milligram protein. Bar heights represent the means, brackets indicate ±SEM. Numbers in or above the bars indicate the number of animals studied in each group. Statistical significance is indicated at the top of the bars.

groups were 1.50 ± 0.24 IU/mg protein (CGS-12970) and 1.56 ± 0.13 IU/mg protein (CGS-16617). However, treatment with a combination of both agents (CGS-12970 + CGS-16617) significantly reduced the loss of CK activity from the ischemic myocardium. The CK value in this group was 0.87 ± 0.23 IU/mg protein and was significantly lower than that of the vehicle group ($P < 0.01$). Thus, the combination of the ACE and the TxA_2 inhibitor was very effective in retarding the extension of infarcted tissue under conditions in which each agent alone was ineffective.

Discussion. In this study, we employed two pharmacologic agents which inhibit the synthesis of two separate vasoactive mediators of myocardial ischemia, angiotensin II and TxA_2. These agents were studied separately and in combination during acute myocardial infarction. The renin–angiotensin system and the eicosanoid system (e.g., TxA_2), in addition to being two potent vasoactive mediator systems, may also have important interrelationships. In this regard, sufficient data are available to suggest that a mechanism involving vasodilator eicosanoids (e.g., prostacyclin) both curtails the pressor phase and mediates the depressor phase of the hemodynamic responses to angiotensin II (22). Moreover, specific inhibition of TxA_2 formation has been shown to reorient endoperoxide precursors toward the generation of prostacyclin and other vasodilator prostaglandins (23) and, thus, addition of a thromboxane synthetase inhibitor may result in attenuation of the vascular responses to angiotensin II and may augment the effects of an ACE inhibitor on the peripheral vasculature.

Angiotensin II-induced vasoconstriction can enhance local conditions favoring additional TxA_2 release. Furthermore, angiotensin II is an effective stimulus for eicosanoid synthesis in several tissues (22) and local release of TxA_2 in the kidney by angiotensin II has recently been suggested (24). Addition of ACE inhibitors which block the synthesis of angiotensin II might thus reduce local TxA_2 production and could facilitate the effect of a thromboxane synthetase inhibitor.

In a previous study (17), we found that CGS-16617 employed at the same dose (1 μg/kg) markedly attenuated the angiotensin I-induced pressor response without exerting direct effects on blood pressure or heart rate, indicating that this dose effectively inhibits the conversion of angiotensin I to angiotensin II in rats without altering myocardial oxygen demand. In the same study, CGS-16617 de-

creased proteolytic activity *in vitro* in pancreatic homogenates. Addition of CGS-16617 and a TxA_2 synthetase inhibitor reduced the proteolytic activity even further than each agent given alone. This antiproteolytic action may retard the autolytic processes participating in the extension of myocardial ischemic damage. In this regard, protease inhibitors have been found to limit the extension of ischemic damage in acute myocardial ischemia (25).

In the present study, treatment with either CGS-12970 or CGS-16617, at the doses employed, did not have a significant effect upon the ischemic damage in the rat myocardium 48 hr after acute coronary artery ligation. However, administration of both drugs significantly attenuated CK loss from the LVFW. Since this effect was present 48 hr after coronary artery ligation, when the necrotic process is at its peak (3), our findings suggest that the combined treatment is superior to each drug alone in reducing the infarcted tissue mass at this time. These data suggest an interaction between the renin–angiotensin and the eicosanoid systems in the pathogenesis of acute myocardial ischemia and indicate that combined inhibition of angiotension II and TxA_2 formation may be a useful approach to the treatment of acute myocardial infarction.

1. Ertl G, Alexander RW, Kloner RA. Interactions between coronary occlusion and the renin–angiotension system in the dog. Basic Res Cardiol **78**:518–533, 1983.
2. Koch-Weser J. Nature of the inotropic action of angiotensin on ventricular myocardium. Circ Res **16**: 230–237, 1965.
3. Hock CE, Ribeiro LGT, Lefer AM. Preservation of ischemic myocardium by a new converting enzyme inhibitor, enalaprilic acid, in acute myocardial infarction. Amer Heart J **109**:222–228, 1985.
4. Ertl G, Kloner RA, Alexander RW, Braunwald E. Limitation of experimental infarct size by an angiotensin converting enzyme inhibitor. Circulation **65**: 40–48, 1982.
5. Van Gilst WH, De Graeff PA, Wesseling H, De Langen CDJ. Reduction of reperfusion arrhythmias in the ischemic isolated rat heart by angiotensin converting enzyme inhibitors: A comparison of captopril, enalapril and HOE-498. J Cardiovasc Pharmacol **8**:722–728, 1986.
6. De Graeff PA, Van Gilst WH, De Langen CDJ, Kingma JH, Wesseling H. Concentration dependent protection by captopril against ischemia-reperfusion injury in the isolated rat heart. Arch Int Pharmacodyn Ther **280**:181–193, 1986.
7. Pfeffer MA, Pfeffer JN, Steinberg C, Finn P. Survival after an experimental myocardial infarction: Beneficial effects of long-term therapy with captopril. Circulation **72**:406–412, 1985.
8. Hamberg M, Svensson J, Samuelsson B. Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA **72**:2994–2998, 1975.
9. Lefer AM, Smith EF III, Araki H, Smith JB, Aharony D, Claremon DA, Magolda RL, Nicolaou KC. Dissociation of vasoconstrictor and platelet aggregatory activities of thromboxane by carboxylic thromboxane A_2, a stable analog of thromboxane A_2. Proc Natl Acad Sci USA **77**:1706–1710, 1980.
10. Lewy RI, Wiener L, Walinsky P, Lefer AM, Silver MJ, Smith JB. Thromboxane release during pacing induced angina pectoris: Possible vasoconstrictor influence on the coronary vasculature. Circulation **61**: 1165–1171, 1980.
11. Burke SE, DiCola G, Lefer AM. Protection of ischemic cat myocardium by CGS-13080, a selective potent thromboxane A_2 synthesis inhibitor. J Cardiovasc Pharmacol **5**:842–847, 1983.
12. Hock CE, Lefer AM. CGS-12970, a thromboxane synthetase inhibitor, limits ischemic damage following coronary artery occlusion. Res Commun Chem Pathol Pharmacol **52**:285–294, 1986.
13. Brezinski ME, Yanagisawa, Darius H, Lefer AM. Antiischemic actions of a new thromboxane receptor antagonist during acute myocardial ischemia in cats. Amer Heart J **110**:1161–1167, 1985.
14. Schrör K, Thiemermann C. Treatment of acute myocardial ischemia with a selective antagonist of thromboxane receptors (BM 13177). Brit J Pharmacol **87**: 631–637, 1986.
15. Lehmann M, Norman J, Zimmerman MB, Barclay B, Goodman FR. Comparative effects of angiotensin converting enzyme (ACE) inhibitors on tissue ACE activity and blood pressure in the conscious spontaneously hypertensive rat (SHR). Pharmacologist **27**: 192, 1985.
16. Selye H, Bajusz E, Grasso S, Mandell P. Simple techniques for the surgical occlusion of coronary vessels in the rat. Angiology **11**:398–407, 1960.
17. Bitterman H, Philips, GR III, Dragon G, Lefer AM. Potentiation of the protective effects of a converting enzyme inhibitor and a thromboxane synthetase inhibitor in hemorrhagic shock. Fed Proc **46**:1264, 1987.
18. McLean D, Fishbein MC, Braunwald E, Maroko PR. Long-term preservation of ischemic myocardium after experimental coronary artery occlusion. J Clin Invest **61**:541–551, 1977.
19. Roberts R, Ishikawa Y. Enzymatic estimation of in-

farct size during reperfusion. Circulation **68**:183–190, 1983.
20. Rosalki SB. An improved procedure for serum creatinine phosphokinase determination. J Lab Clin Med **96**:696–705, 1967.
21. Gornall AG, Bardowill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem **177**:751–766, 1949.
22. Rowe BP, Nasjletti A. Biphasic blood pressure responses to angiotensin II in the conscious rabbit: Relation to prostaglandins. J Pharmacol Exp Ther **225**: 559–563, 1983.
23. Vermylen J, Deckmyn H. Reorientation of prostaglandin endoperoxide metabolism by a thromboxane synthetase inhibitor: In vitro and clinical observations. Brit J Clin Pharmacol **15**:17S–22S, 1983.
24. Branch RA, Goto F, Herzer W, Jackson EK. Angiotensin II induced release of thromboxane A_2: Possible role in mediating the effects of angiotensin II on glomerular filtration in the hypoperfused canine kidney. Sixth International Conf Prostag Related Compounds, Florence, Italy, June 3–6, 1986.
25. Lefer AM, Spath JA. Preservation of myocardial integrity by a protease inhibitor during acute myocardial ischemia. Arch Int Pharmacodyn Ther **211**:225–236, 1974.

Received December 18, 1986. P.S.E.B.M. 1987, Vol. 185.
Accepted March 26, 1987.