Plasma Bradykinin and Prostaglandin Metabolism and Exercise Testing in Patients with Silent Myocardial Ischemia Compared with Patients with Painful Myocardial Ischemia

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Bradykinin, alone or in combination with prostaglandin, is thought to produce pain in patients with coronary heart disease. To elucidate this further, we have investigated and compared serum bradykinin, TXB₂ and 6 KPGF_{1α} levels in patients with silent myocardial ischemia (SMI, n = 18), painful myocardial ischemia (PMI, n = 8) and normal subjects (NL, n = 18). In addition, SMI and PMI subjects were given exercise testing and the results then compared.

After Holter monitoring for 48 hours, exercise testing was performed. Blood was sampled in the morning between the Holter and exercise regimen.

Maximal heart rate, systolic blood pressure and the double products were not significantly different between the SMI and PMI groups. The duration of exercise for the SMI group was 7.08 ± 2.1 min vs 5.9 ± 1.9 in the PMI group ($p < 0.10$). Plasma bradykinin was 14 ± 3 pg/ml in the SMI group and 15 ± 3 in the PMI group (N.S), whereas it was 7 ± 4 in the NL ($p < 0.05$). The TXB₂/6KPGF_{1α} for the SMI group was 1.3 ± 0.3 , which was significantly higher than that for the NL group (0.8 ± 0.3 , $p < 0.01$), though this did not greatly differ from the PMI group (1.2 ± 0.3).

These results suggest that SMI patients under Holter monitoring who manifest no symptoms but show significant ST segment depressions must receive the same careful attention given to PMI patients. In both group of patients bradykinin and prostaglandin metabolism is similarly changed, as was demonstrated by exercise stress testing.

SILENT myocardial ischemia (SMI) is recognized as an important clinical entity in patients with a coronary artery disease (CAD).¹⁻³ Recent studies have suggested that the absence of chest pain during an ischemic episode may indicate that its occurrence may be due to

hormonal, neural, and psychological factors.⁴ Although bradykinin is a potent pain producing substance which has been reported to be elevated in patients during an attack of angina or an acute myocardial infarction,^{5,6} there have been no reports concerning bradykinin levels in the patients with SMI.

We have studied the levels of bradykinin in patients with SMI and also the plasma prostaglandin metabolism, since bradykinin is recognized as being in the same metabolic category

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Fig.1. Plasma bradykinin level and TXB₂/6ketoPGF_{1α} ratio in patients with SMI, PMI and NL group.

as prostaglandin.⁷ We also investigated whether prostaglandin metabolism plays an important role in the pathogenesis of myocardial ischemia.

MATERIALS AND METHODS

Our investigations involved 44 patients divided into three groups. Group I consisted of 18 patients (mean age: 58 years) who had at least 75% or greater luminal narrowing of at least one major coronary artery, and had undergone a positive exercise test. Patients in this group had shown no anginal symptoms, but had experienced an ST segment depression of at least 30 sec by Holter monitoring. Group II consisted of 10 patients (mean age: 56 years) who had similar stenotic lesions, though less than 75%, in their major coronary arteries, and had undergone a positive exercise test. This group had exhibited painful myocardial ischemia (PMI) in their clinical history and also during Holter monitoring. Group III were our controls, 16 healthy volunteers (mean age: 25 years).

Noninvasive Testing

Ambulatory electrocardiographic monitoring (Holter) was performed using a Marquette's two channel recorder, with a frequency response of

0.50 to 100 HZ meeting the American Heart Association standards. Patients underwent 48 hours of monitoring prior to the start of the exercise test. Analysis of ST segment was done using an 8000T analyzing system. The reliability of our measurements of these segments was confirmed using a conventional 12 leads electrocardiographic recording. The ST segment was positive in the Holter monitoring system when the electrocardiographic strips showed a horizontal or downsloping ST segment depression of 1 mm after the J point that lasted for 60 msec.

Treadmill testing was performed after Holter monitoring, using the standard Bruce protocol, with 12 leads electrocardiograms taken before, during, immediately after exercise, and 1, 3 and 5 min after exercise. ST changes that were due to hyperventilation or a shift in position were carefully excluded. Patients who showed any electrocardiographic abnormalities such as an ST segment depression with or without left ventricular hypertrophy or bundle branch block or pre-excitation syndrome were also excluded from the study. A positive response was defined as showing a horizontal or downsloping ST segment depression of at least 1 mm after the J point for 80 msec.

TABLE 1 COMPARISON OF EXERCISE TESTING IN PATIENTS WITH SMI AND PMI

	HR		BP		PRP		Ex Time
	Rest	Max	Rest	Max	Rest	Max	
SMI	70 ± 17	120 ± 17	132 ± 18	169 ± 23	95 ± 37	202 ± 37	7.08 ± 2.1
PMI	66 ± 8	118 ± 18	138 ± 17	160 ± 15	92 ± 19	189 ± 40	5.94 ± 1.8
p	NS	NS	NS	NS	NS	NS	NS

HR = heart rate; BP = blood pressure; PRP = pressure rate product

Fig.2. Records of Holter monitoring and treadmill testing in patients with SMI. Note that ST segment depression by treadmill testing (right side) without chest pain is similarly changed in the record of Holter monitoring (left and middle) during treadmill testing and bicycle riding. Plasma bradykinin, TXB₂/6ketoPGF_{1α} levels were shown in the lower part of this figure.

Invasive Testings

Coronary arteriography was performed in a standard manner at least 4 weeks prior to the

noninvasive tests with the results read by two observers. A significant lesion was considered to be one that occluded a major coronary artery or

Fig.3. Records of Holter monitoring and treadmill testing in patients with PMI. Note that ST segment depression by treadmill testing (right side) during chest pain is similarly changed in the record of Holter monitoring (left middle). Plasma bradykinin, TXB₂/6ketoPGF_{1α} were shown in the lower part of this Figure.

one of its large branches by at least 75%, as has been previously described.

Blood Sampling Collection and Assay

Before treadmill testing, while the subjects were still fasting, blood specimens were taken from the antecubital vein. The first 0.5 ml of blood was discarded, leaving approximately 10 ml for assay. Thromboxane B₂ (TXB₂) was measured by radioimmunoassay of a 2 ml blood sample in 0.5 ml of 4.5 mM EDTA and 1 mM acetylsalicylic acid solution, to prevent in vitro breakdown of the arachidonic acid and prostaglandin release. ³H, standard TXB₂ and its antigen were obtained from the New England Nuclear Co., Ma. Prostaglandin F_{1α} (6KPGF_{1α})

was measured as a stable product of PGI₂ by radioimmunoassay using a standard antigen to 6 keto PGF_{1α} obtained from the New England Nuclear Co., Ma.⁸ Bradykinin was measured by radioimmunoassay of a 2 ml blood sample.⁹ These blood samples were mixed and immediately placed on ice for no longer than 30 min before centrifugation at 400G for 20 min at 4°C, then stored at -80°C. All assays had a 95% recovery efficiency.

Statistical analysis was carried out using Student's t-test. Unpaired t test was used to compare changes between normal subjects and patients, and was confirmed the method of Bonferroni.⁹ Paired t tests were used to compare

changes in patients.

RESULTS

Bradykinin and TXB₂/6KPGF_{1α} (Fig. 1)

The plasma levels of circulating bradykinin did not differ significantly between patients with SMI and PMI. However the level in the normal control group was 7 ± 4 pg/ml, which was lower than that in the SMI group (14 ± 3) and in the PMI group (15 ± 3). With regard to the prostaglandin metabolism, TXB₂/6KPGF_{1α} in the SMI group was 1.4 ± 0.3 , which was significantly higher than in the control group (0.8 ± 3) ($p < 0.01$), whereas the TXB₂/6KPGF_{1α} of the PMI group was 1.2 ± 0.3 ($p < 0.1$, vs the control group).

Exercise Testing and Ambulatory Monitoring (Table I, Fig. 2 & 3).

These two tests were limited to the SMI and PMI groups since the parameter in normal controls was expected to differ significantly from the other two groups. In all patients a 12 lead electrocardiogram was recorded and none showed a significant ST segment elevation (more than 1 mm at the J point) at any lead while at rest. The mean $\pm$ standard deviation of the exercise time was 5.9 ± 1.8 min for the SMI group and 7.08 ± 2 for the PMI group ($p < 0.1$). The maximal heart rate was 120 ± 17 beats/min in the SMI group and 118 ± 18 in the PMI group (NS), whereas the mean maximal ST segment depression was 2.3 ± 0.5 mm in the SMI group and 2.4 ± 0.3 in the PMI group (NS). Also, no appreciable differences were noted in the systolic blood pressure and the double product between the groups while at rest or during exercise.

DISCUSSION

Our subjects were shown to have a similar history of angina pectoris, similar coronary arteriographic findings, and all had undergone a positive exercise test. Patients in both groups were able to complete their exercise test and achieve a heart rate of approximately 120 beats per minute. Further, to insure accuracy, we eliminated the possible technical problems that might have occurred because of abnormal ST segment changes that might be due to hyperventilation or a shift in position. In addition, we confirmed the close correlation of the Holter monitoring and the conventional 12 lead electro-

cardiogram, assuring the reliability of our results.

It is commonly known that when a significant transient imbalance in oxygen supply and demand develops in the coronary circulation, corresponding characteristic changes are seen in the electrocardiogram, in hemodynamic variables, in segmental or global ventricular functions and in metabolism.¹¹⁻¹² Previous studies have suggested that one of the possible causes of myocardial ischemic pain is the appearance of beta-endorphin¹³⁻¹⁵ which is one of the most potent of endogenous opioidlike peptides and is believed to play an important role in the endogenous analgesic system. Similarly, more than a decade ago, bradykinin was said to produce pain, in addition to the role that it plays in smooth muscle contraction, vasodilation, increase in capillary permeability and bronchial muscle contraction.¹⁶

Kimura et al⁵ shown that bradykinin was elevated after the ligation of the coronary artery and Hashimoto et al⁶ confirmed the elevation of the plasma bradykinin level in the blood of patients with angina pectoris or in those who had an acute myocardial infarction. However they did not measure the level of prostaglandin metabolism simultaneously. Needleman et al⁷ suggested that bradykinin has a relationship with prostaglandin in the heart.

In appropriate cardiac oxygen demand due to various endogenous or exogenous stimuli usually causes a regional ischemia that activates kinin synthesis and in turn, stimulates local prostaglandin production. This, alone or in combination with kinins, causes pain and initiates afferent chemoreflexes.

Therefore, to show this as a thesis would require measurement of the plasma prostaglandin level, and the determination of its relationship to the plasma bradykinin level. In this study we found that both groups showed a similarly elevated plasma bradykinin level. Further, TXB₂/6KPGF_{1α} also showed similarly higher values in both groups than in the control group. Our results lead to two speculations: that bradykinin is not the only substance that produces pain, and that bradykinin may only elevate at the moment the patient feels pain. The latter is difficult to prove by a clinical study, since the half life of the bradykinin is too short.

In conclusion, our results show that when the ST segment decreases to 1 mm for 30 sec or more during Holter monitoring, even patients who manifest no symptoms should be treated in

the same manner as patients manifesting symptoms, because both bradykinin and prostaglandin metabolism is similarly changed, as was demonstrated by exercise stress testing.

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