Coronary Spasm, Prostaglandin and HLA Factors

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To elucidate the contribution of prostanoids in coronary spasm, plasma levels of thromboxane B₂ (TXB₂) and 6-keto PGF₁α at the coronary sinus and ascending aorta in 21 patients with variant angina were measured, as compared with findings in 20 with effort angina and 13 subjects with normal coronaries. In the coronary sinus blood, plasma TXB₂ in patients with effort angina exhibited statistically significant high levels, as compared with data in the controls. On the contrary, the data obtained from patients with variant angina were not statistically significant. However, eight patients whose coronary angiogram revealed more than 50% of coronary stenoses had statistically significant high levels of TXB₂ and other patients with normal coronaries or less than 50% of narrowing showed almost the same levels of TXB₂ as the controls.

In contrast to TXB₂, the plasma levels of 6-keto PGF₁α in patients with variant angina were very low in both groups with variant angina. These data suggest that high levels of TXB₂ observed in patients with atherosclerotic coronaries may be an accelerating factor while low levels of prostacyclin may be an essential factor leading to spasm.

HLA analysis of 23 patients with variant angina was performed to search for genetic factors, under the hypothesis that such may contribute to the low levels in prostacyclin. This preliminary study revealed statistically significant high frequencies of Bw52 and B-40 in the patients, as compared with frequencies among 152 normal Japanese. Genetic studies are ongoing in our clinic.

CORONARY spasm is an event related to ischemic heart disease. Although coronary spasm has generally been considered to be responsible for the variant form of angina pectoris, a keen interest has been directed to the roles of stenoses in the pathophysiology of myocardial infarction and angina on exertion. Pharmacodynamic and/or biochemical approaches have been used in attempts to elucidate the mechanisms involved in coronary spasm. McAlpin proposed that eccentric subintimal plaques can theoretically potentiate the effect of arterial smooth muscle contraction by acting as levers. Nanda and Henry reported an increased number of serotonin and α-adrenergic receptors in atherosclerotic lesions of the vessel wall of rabbits. However, the mechanisms related to spasm are still poorly understood and various hypotheses have been reported.

PROSTANOIDS AND VARIANT ANGINA

The discovery of thromboxane A₂ (TXA₂) in 1974 and clarification of its physiological function together with the discovery of prostacyclin (PGI₂) in 1975 clearly demonstrated the importance of the role of prostaglandins in the exquisite balance between the blood flow

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6-keto PGF₁α
Genetic factor
HLA

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and the vessel wall. Any alteration in this balance leads to various diseases of vascular wall, in particular atherosclerotic and thrombotic disorders\(^\text{20-24}\) Since Ellis et al. suggested in 1976 the roles of TXA\(_2\) on coronary smooth muscle contraction\(^\text{25}\) prostanoids have gained attention as important local hormonal substances which modulate coronary circulation\(^\text{26-29}\) A disequilibrium in the levels of these substances would lead to anginal attacks or even to myocardial infarction in patients with coronary heart disease\(^\text{30-34}\) Tada et al. noted the increased plasma levels of TXA\(_2\) in patients with variant angina and stressed that transient coronary vasospasm might be induced by TXA\(_2\), locally released in coronary circulation by the aggregating platelets\(^\text{35,36}\) They found a positive correlation between plasma levels of TXB\(_2\) and the threshold dose of ergonovine which induced vasospasm.

Lewy et al. also reported the high levels of TXB\(_2\) in patients with variant angina and found statistically increased levels during attack, as compared with findings in patients with coronary arterial disease\(^\text{37,38}\)

Lewis et al.\(^\text{39}\) and Mehta et al.\(^\text{28}\) observed the same responses of prostanoids in patients with variant angina as did Lewy et al.\(^\text{37,38}\) and increases in thromboxane A\(_2\) thus seemed to be an important causative factor in the induction of vasospasm.

On the other hand, Chierchia et al. found that neither PGI\(_2\) nor a thromboxane inhibiting agent prevented clinical vasospastic episodes, thereby suggesting the lack of an important role of prostanoids in coronary vascular spasm\(^\text{30,41}\) Robertson et al. found that, despite increased levels of TXB\(_2\) in plasma of patients with variant angina

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during attack, neither aspirin nor indomethacin has any effect on the frequency or duration of ischemia and suggested that increases in levels of TXB₂ are not the cause but rather the result of spasm. Sobel et al. reported the negative role of thromboxane in coronary spasm.

These conflicting data on the roles of prostanoids in coronary spasm suggested to us that changes in prostanoids in patients with variant angina should be compared with levels in patients with effort angina, at a time when they were free from attack.

**Materials and Methods**

The subject studied included twenty-one Japanese men and women ranging in age from 46–65 years with the variant form of angina pectoris (VA) and twenty with effort angina (EA). Thirteen age matched subjects, as controls, were all confirmed angiographically to have normal coronaries. The patients with variant angina all had episodes of anginal attacks at rest or after alcohol ingestion and ST elevation in the electrocardiogram was confirmed during the attacks. Coronary spasm was also confirmed angiographically by the injection of 0.2 mg of ergonovine maleate.

All patients with effort angina were diagnosed according to exertional anginal attack documented in their medical records, and the positive changes seen on the electrocardiogram during the treadmill exercise test. The angiogram showed over 75% of obstructive atherosclerotic lesions. A “negative reaction” with 0.2 mg of ergonovine
injection was also confirmed. Five ml of blood samples were taken at the coronary sinus and ascending aorta from all patients and controls. The samples were immediately placed in heparinized plastic tubes containing 0.1 ml of $10^{-2}$ M indomethacin (Merck Co.) solution and centrifuged at 3,000 rpm for 10 min at 0°C. Plasma thus obtained was kept frozen at −80°C until analysis. Plasma levels of thromboxane B$_2$ (TXB$_2$) and 6-keto prostaglandin F$_{1\alpha}$ (6-keto PGF$_{1\alpha}$) were measured by radioimmunoassay using $^{125}$I-TXB$_2$-tyramide and $^{125}$I-6-keto PGF$_{1\alpha}$-tyramide$^{44-46}$. To study the relationships between prostanoids and coronary atherosclerosis, patients with variant angina were subdivided into two groups, thirteen patients whose coronary angiogram revealed normal or less than 50% luminal diameter narrowing (VA(−)) and eight patients with greater than 50% fixed narrowing (VA(+)).

**Results**

Figure 1 summarizes the plasma levels of TXB$_2$ in the 3 groups. In the coronary sinus samples, TXB$_2$ levels of plasma in patients with effort angina were 438 ± 66 pg/ml, a statistically significant high level as compared with 200 ± 32 in the normal controls ($p < 0.01)$. On the contrary, though patients with variant angina showed a rather high level of TXB$_2$ (304 ± 51), there was no statistically significant different level of TXB$_2$ in plasma between those with VA and the normal controls. However, compared with TXB$_2$ levels in subdivided groups of VA(+) and VA(−), the average level of TXB$_2$ in VA(+) patients was 453 ± 72, such being almost the same level as in patients with effort angina and with a statistically significant high level as compared with data in controls ($p < 0.01$). On the other hand, the TXB$_2$ level in the VA(−) group was 212 ± 58, almost the same as those in normal controls. In samples taken from the aorta, the same changes observed in coronary sinus blood were recognized, with no statistically significant difference.

As shown in Fig. 2, plasma levels of 6-keto PGF$_{1\alpha}$ in those with variant angina were very low (98 ± 17 pg/ml), as compared with 232 ± 59 in the normal controls or 383 ± 120 with effort angina. It should be noted that, in contrast to TXB$_2$ both VA(+) (111 ± 42) and VA(−) (90 ± 13) revealed low levels of 6-keto PGF$_{1\alpha}$. In particular, statistically significant low levels were confirmed in subgroup VA(−), as compared with findings in the normal controls ($p < 0.05$) and also statistically significant low levels of 6-keto PGF$_{1\alpha}$ was noted in aortic samples in cases of variant angina (90 ± 18), as compared with 160 ± 33 in the normal controls ($p < 0.05$).

**Discussion**

In these studies, the following two points should be emphasized. First, thromboxane A$_2$ may be an important enhancing factor, but not an absolute condition for the occurrence of coronary vascular spasm. Thromboxane A$_2$ is a potent contracting substance of smooth muscle cell, and is readily produced and released from platelets locating on the rough surface of the atherosclerotic lesions$^{15,16}$. Actually, patients with ischemic heart disease showed a high level of plasma TXB$_2$ and a concomitant increase during exercise and/or spastic attack$^{45,47}$. The association between coronary arterial spasm and atherosclerotic lesions has been studied since Oster's early description$^{8,13,48,49}$. Recently Shimokawa et al. induced coronary spasm at the area of atherosclerotic lesions in miniature pigs fed a high cholesterol diet after arterial balloon- ing$^{50}$. They concluded that atherosclerosis was a primary causative factor of coronary spasm. Kawachi et al. observed selective hypercontraction in the atherosclerotic area of the canine coronary artery in vivo and proposed that there are hypersensitive smooth muscles in atherosclerotic arteries that are sensitive to ergonovine and serotonin$^{50}$. Henry and Yokoyama also reported an increased sensitivity for ergonovine in atherosclerotic aortic strips$^{52,53}$. Kishi and Numano observed these same changes when atherosclerotic aortic strips were exposed to serotonin$^{54}$. Nanda and Henry confirmed the increased number of serotonergic and alpha adrenergic receptors in aorta from rabbits fed a high cholesterol diet$^{12}$.

Though all these data suggest a close relationship between atherosclerotic lesions and coronary spasm, clinical pathophysiological conditions of variant angina are not well understood. About 10% of patients show no evidence of atherosclerosis angiographically$^{55-57}$. Furthermore, patients with variant angina are few, as compared with the increasing number of patients with coronary atherosclerosis. The mechanism related to spontaneous remission of spasm and exacerbations of symptoms in the absence of obvious changes in coronary artery is unknown. In our patients, 13 of 21 revealed angiographically normal coronary arteries of less than 50% of narrowing and
<table>
<thead>
<tr>
<th>Patients</th>
<th>yrs/sex</th>
<th>HLA</th>
<th>Coronary arteries</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 M.M.</td>
<td>46/F</td>
<td>26</td>
<td>W52 W4</td>
</tr>
<tr>
<td>2 B.S.</td>
<td>63/M</td>
<td>-</td>
<td>W6 40 (W60) W39</td>
</tr>
<tr>
<td>3 M.H.</td>
<td>68/M</td>
<td>2</td>
<td>W6 40 (W60) W54</td>
</tr>
<tr>
<td>4 S.J.</td>
<td>43/M</td>
<td>11</td>
<td>W4 W6</td>
</tr>
<tr>
<td>5 I.M.</td>
<td>57/M</td>
<td>11</td>
<td>W52 W4 W6 40 (W61)</td>
</tr>
<tr>
<td>6 S.H.</td>
<td>62/M</td>
<td>2</td>
<td>W6 39, W62 W4 W7</td>
</tr>
<tr>
<td>7 T.T.</td>
<td>62/M</td>
<td>-</td>
<td>W6 40 (W61) W62</td>
</tr>
<tr>
<td>8 Y.T.</td>
<td>63/M</td>
<td>2</td>
<td>W52 W4 W6 40 (W60)</td>
</tr>
<tr>
<td>9 M.Y.</td>
<td>70/M</td>
<td>-</td>
<td>W52 W4</td>
</tr>
<tr>
<td>10 O.T.</td>
<td>53/M</td>
<td>-</td>
<td>W52 W4</td>
</tr>
<tr>
<td>11 O.U.</td>
<td>64/F</td>
<td>2</td>
<td>W6 39, - W7</td>
</tr>
<tr>
<td>12 M.K.</td>
<td>61/M</td>
<td>-</td>
<td>W52 W4 W6 40 W3</td>
</tr>
<tr>
<td>13 S.T.</td>
<td>56/F</td>
<td>2</td>
<td>W6 40 (W60) W1</td>
</tr>
<tr>
<td>14 H.S.</td>
<td>70/M</td>
<td>2</td>
<td>W52 W4 W6 W60</td>
</tr>
<tr>
<td>15 O.T.</td>
<td>60/M</td>
<td>-</td>
<td>W4 W46, W53 W1 W3</td>
</tr>
<tr>
<td>16 R.Y.</td>
<td>60/M</td>
<td>26</td>
<td>W6 W62 W54 W1 W3</td>
</tr>
<tr>
<td>17 S.S.</td>
<td>60/M</td>
<td>2</td>
<td>W4 W6 13, W63 W3</td>
</tr>
<tr>
<td>18 O.S.</td>
<td>64/M</td>
<td>2</td>
<td>W33 W4 W6 40 (W61)</td>
</tr>
<tr>
<td>19 O.T.</td>
<td>49/M</td>
<td>2</td>
<td>W33 W52 W4 W6</td>
</tr>
<tr>
<td>20 T.I.</td>
<td>53/M</td>
<td>26, W33</td>
<td>W6 40 (W61) -</td>
</tr>
<tr>
<td>21 T.T.</td>
<td>50/M</td>
<td>2</td>
<td>W6 40 (W61) W3</td>
</tr>
<tr>
<td>22 K.T.</td>
<td>54/M</td>
<td>2</td>
<td>W6 40 (W60) W39</td>
</tr>
<tr>
<td>23 N.T.</td>
<td>51/F</td>
<td>2</td>
<td>W6 7, W62 W3 W7</td>
</tr>
</tbody>
</table>
the plasma levels of TXB$_2$ were as low as in the normal controls. Therefore, the high levels of TXB$_2$ shown in cases of variant angina are probably not a contributing factor but rather the result of coronary spasm. The low levels of 6-keto PGF$_{1\alpha}$ were marked in patients with normal coronaries and variant angina. The decrease of PGI$_2$ in smooth muscle cells is one condition during which vasospasm occurs$^{58,59}$ Dembinska-Kiełk et al. reported a decrease of PGI$_2$ in atherosclerotic coronaries of the rabbit$^{60}$ and De Angelo et al. confirmed the low levels of PGI$_2$ in the atherosclerotic human aorta, compared with findings in the normal aorta$^{61}$ This may explain why in atherosclerotic portions of the coronary arteries spasm is easily provoked. Shimokawa et al. also mentioned that indomethacin treatment intensified coronary spasm, as induced by histamine$^{62}$ Kishi and Numano observed the increased sensitivities of coronaries of swine to ergonovine and serotonin and which were preincubated in media mixed containing $10^{-8}$M of aspirin for 60 min, as compared with of control tissues incubated without aspirin. In the media containing aspirin, 6-keto PGF$_{1\alpha}$ was absent while media without aspirin contained 0.68 pg/ml of 6-keto PGF$_{1\alpha}$. Miwa et al. reported interesting clinical cases in which patients with variant angina given 4 g/day of aspirin, readily experienced spastic attacks during the exercise test$^{53,64}$ All these data indicate that a low level of 6-keto PGF$_{1\alpha}$ may be a more important causative factor in coronary spasm than previously acknowledged.

### HLA AND VARIANT ANGINA

Nevertheless, it is unclear why low levels of 6-keto PGF$_{1\alpha}$ occur in patients with variant angina, especially in those with normal coronaries. Though many patients have coronary atherosclerosis, why do so few experience coronary spasm? One observation is that the frequencies of variant angina seem to differ among races. There are comparatively many cases of variant angina in Japan and Italy, as compared with countries where coronary atherosclerosis is more relevant disease$^{65}$ Therefore, the genetic factors contributing to this condition were

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**Table II: Frequencies of HLA Antigens in Patients with Variant Angina**

<table>
<thead>
<tr>
<th>HLA Antigens</th>
<th>Variant Angina (N: 23)</th>
<th>Control % (N: 152)</th>
<th>$\chi^2$ Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>A - 2</td>
<td>56.5</td>
<td>41.4</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>21.7</td>
<td>13.2</td>
<td></td>
</tr>
<tr>
<td>w24</td>
<td>56.5</td>
<td>55.9</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>21.7</td>
<td>25.7</td>
<td></td>
</tr>
<tr>
<td>w33</td>
<td>13.0</td>
<td>12.5</td>
<td></td>
</tr>
<tr>
<td>B - w 4</td>
<td>52.2</td>
<td>57.6</td>
<td>$^*\chi^2 = 3.86$ (p &lt; 0.05) (rr = 2.40)</td>
</tr>
<tr>
<td>w 6</td>
<td>78.3</td>
<td>87.8</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>26.1</td>
<td>16.6</td>
<td></td>
</tr>
<tr>
<td>40*</td>
<td>47.8</td>
<td>29.0</td>
<td></td>
</tr>
<tr>
<td>w44</td>
<td>17.4</td>
<td>13.1</td>
<td>$^{**}\chi^2 = 4.39$ (p &lt; 0.04) (rr = 2.71)</td>
</tr>
<tr>
<td>w52**</td>
<td>34.8</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td>C - w 1</td>
<td>21.7</td>
<td>27.2</td>
<td></td>
</tr>
<tr>
<td>w 3</td>
<td>47.8</td>
<td>54.4</td>
<td></td>
</tr>
<tr>
<td>DR - 2***</td>
<td>52.2</td>
<td>32.4</td>
<td>$^{***}\chi^2 = 2.93$</td>
</tr>
<tr>
<td>4</td>
<td>34.8</td>
<td>29.7</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>13.0</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>w 8</td>
<td>17.4</td>
<td>23.0</td>
<td></td>
</tr>
<tr>
<td>w 9</td>
<td>43.5</td>
<td>29.7</td>
<td></td>
</tr>
<tr>
<td>MT - 1</td>
<td>69.5</td>
<td>65.8</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>56.5</td>
<td>65.8</td>
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<tr>
<td>3</td>
<td>69.6</td>
<td>64.4</td>
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</tr>
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</table>
analysed and compared.

Materials and Methods
Twenty three patients had been diagnosed as having variant form of angina pectoris, based on ECG findings and clinical features. Coronary spasm was confirmed angiographically by ergonovine injection. These patients were all included in prostanoid studies and HLA A, B, C and D loci were studied using 56 antisera and the results were compared with findings in 128 normal Japanese. B cells obtained from patients were typed for DR, MT antigens. The antigenic assignments were based on serologic reactions and conformed to specificities assigned by the Second Asia Oceania HLA Workshop (1981). Comparison of the frequencies was made according to data on normal Japanese reported in the Eighth International Histocompatibility Workshop. A statistical analysis was performed using the chi square test of antigens in association with this disease.

Results
Table I shows HLA typings in A, B, C, DR and MT loci in 23 patients with variant angina and angiographical findings in the coronary arteries and Table II summarizes the frequencies of HLA antigens in these patients, as compared to frequencies in 152 normal Japanese. Statistically significant high frequencies of B-40 and Bw52 were confirmed in this analysis, as compared with those in normal controls. There were no differences among frequencies of HLA antigens between 8 patients with coronary atherosclerosis and 15 with normal coronaries.

Discussion
There is little in the available literature on genetic factors and coronary spasm. Mauritzon et al noted that in the work of Freedman et al spasm was caused by an increased sensitivity of coronary arteries to vasoconstriction. The former reported a clinical trial on 24 siblings of eleven patients with variant angina and checked whether or not coronary spasm could be induced in these 24 siblings by injection of ergonovine. In no individual was spasm provoked. From these results, they concluded that coronary spasm is an acquired disease. The average age of these siblings was 46 years, comparatively young, considering the usual ages of patients with variant angina.

As high frequencies of Bw52 often occur in Japanese or other Asians peoples further related studies are warranted.

Acknowledgement
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