RISK FACTORS FOR CAROTID ATHEROSCLEROSIS
AND PLATELET ACTIVATION

OSAMU UYAMA, M.D., YUKIKO MATSUI, MSc., SOUCHIRO SHIMIZU, M.D.*
HSAMASA MICHISHITA, M.D.* AND MINORU SUGITA, M.D.*

Thromboxane A₂ biosynthesis was studied in healthy subjects, in patients in
whom the extent of carotid atherosclerosis was determined, and in patients re-
ceiving chronic aspirin treatment, to determine what factors activate platelets to
develop carotid atherosclerosis.
Urinary 11-dehydrothromboxane B₂, a major metabolite of thromboxane A₂,
was measured by radioimmunoassay after purification by reverse-phase HPLC.
The extent of carotid atherosclerosis was determined by real-time B-mode ultra-
sonography. The severity of carotid atherosclerosis in each subject was eval-
uated by plaque score, which was computed by summing the maximum thickness
of plaque measured in millimeters.
Urinary excretion of 11-dehydrothromboxane B₂ in healthy subjects was higher
(P<0.01) in cigarette smokers (1063±244 ng/g creatinine) than in non-smokers
(815±183 ng/g creatinine). Aspirin significantly suppressed 11-dehydrothrom-
boxane B₂ excretion (266±114 ng/g creatinine). In the 24 patients in whom the
plaque score was measured, multivariate analysis indicated a significant positive
 correlation between urinary excretion of 11-dehydrothromboxane B₂ and
plaque score, age, smoking and hypercholesteremia.
Our results indicate that risk factors such as age, hypercholesteremia, athero-
sclerosis and smoking activate platelets in vivo to develop carotid atherosclero-
sis.
(Ipn Circ J 1994; 58: 409~415)

THROMBOXANE A₂ (TXA₂), the major cy-
clooxygenase product of arachidonic
acid in platelets, is a potent platelet aggre-
gant1 and its biosynthesis increases in
diseases associated with platelet activa-
tion2~4 Since TXA₂ is a very labile com-
 pound, its hydrolysis product, thromboxane
B₂ (TXB₂), has been widely assayed as a
quantitative index of platelet activation.
However, previous studies which have mea-
sured immunoreactive TXB₂ have been sub-
ject to sampling and analytical errors5 11-
Dehydro-TXB₂ has recently been identified

Key words:
11-Dehydrothromboxane B₂
Carotid Atherosclerosis
Platelet

(Received June 11, 1993; accepted December 25, 1993)
College of Nursing Art and Science, Hyogo
*Fifth Department of Internal Medicine, Hyogo College of Medicine, Nishinomiya, Japan
Mailing address: Osamu Uyama, M.D., Hyogo College of Nursing Art and Science, Hyogo, 13-71 Kitaoji-cho,
Akashi 673, Japan

Japanese Circulation Journal Vol.58, June 1994 409
TABLE I  CHARACTERISTICS OF THE SUBJECTS EXAMINED

<table>
<thead>
<tr>
<th>Group</th>
<th>Sex</th>
<th>Number</th>
<th>Age range</th>
<th>Age mean±SD</th>
<th>Alcohol Intake (g/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>non-smokers</td>
<td>male</td>
<td>15</td>
<td>31–60</td>
<td>48.0±8.1</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>12</td>
<td>38–64</td>
<td>48.5±6.8</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(48.2±7.4)</td>
<td></td>
</tr>
<tr>
<td>cigarette smokers</td>
<td>male</td>
<td>16</td>
<td>38–71</td>
<td>50.8±8.8</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>1</td>
<td>57</td>
<td>57</td>
<td>8</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(51.1±8.6)</td>
<td></td>
</tr>
<tr>
<td>Plaque score-measured</td>
<td>male</td>
<td>19</td>
<td>35–80</td>
<td>61.3±12.7</td>
<td>0</td>
</tr>
<tr>
<td>patients</td>
<td>female</td>
<td>5</td>
<td></td>
<td></td>
<td>24</td>
</tr>
<tr>
<td>Chronic aspirin treatment</td>
<td>male</td>
<td>5</td>
<td>33–68</td>
<td>56.3±11.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>female</td>
<td>2</td>
<td></td>
<td></td>
<td>7</td>
</tr>
</tbody>
</table>

( ): mean±SD of both sexes

by B-mode ultrasonography, was significantly higher than those in healthy subjects and patients with cerebral infarction who had no distinct carotid lesion? However, in that study carotid atherosclerosis was assessed qualitatively by ultrasonography.

In the present investigation, high-resolution real-time B-mode ultrasonography was performed to determine the extent of carotid atherosclerosis, and the results were quantified by using a scoring system. The aim of this study was to determine which factors (carotid atherosclerosis, age, male sex, hypercholesteremia, hypertension, diabetes mellitus, obesity, and cigarette smoking) were related to urinary 11-dehydro-TXB₂ excretion, a quantitative index of platelet activation, by using multivariate analysis.

SUBJECTS AND METHODS

Forty-four healthy subjects (27 non-smokers and 17 cigarette smokers), who underwent a health examination to screen for adult diseases (atherosclerotic diseases, hypertension, diabetes mellitus, hepatitis, and heart diseases), and who were shown not to have these diseases, were studied to ascertain the normal value of urinary 11-dehydro-TXB₂ excretion. All medications were discontinued at least 2 weeks before the study. Low-density lipoprotein cholesterol (LDL-chol) was determined by the equation proposed by Friedewald et al \( (\text{LDL}-\text{chol} = \text{total cholesterol} - \text{high-density lipoprotein cholesterol} - 1/5 \text{ triglyceride})^{10} \). The atherogenic index was calculated as the ratio of \((\text{total cholesterol} - \text{HDL}-\text{chol})\) and \(\text{HDL}-\text{chol}\).

Since HDL-chol levels have been shown to be positively associated with alcohol consumption\(^{11}\) the subjects were classified by alcohol intake into two categories: non-heavy-alcohol drinkers (up to 40 g alcohol per day) and heavy-alcohol drinkers (40 g or more per day). One large bottle (633 ml) of beer, 180 ml of Japanese sake and one double cup of whisky each contain approximately 23 g of alcohol.

Seven patients (4 with cerebral infarction, 2 with transient ischemic attack, 1 with old myocardial infarction) who were receiving chronic aspirin treatment (100 mg/day) were also studied. Cigarette smokers were consisted of current smokers who used more than 5 cigarettes per day. Non-smokers consisted of both non-smokers and former smokers who had not smoked for more than 6 months. The body mass index (kg/m²) of each subject was determined from their height and body weight. Finally, 24 patients were examined to evaluate the extent of carotid atherosclerosis. The characteristics of the subjects in each of the study groups.
TABLE II CLINICAL DATA FOR 24 PATIENTS IN WHOM THE PLAQUE SCORE OF CAROTID ARTERY WAS MEASURED

<table>
<thead>
<tr>
<th>Pt/Sex/Age</th>
<th>Disease</th>
<th>Smoking</th>
<th>11-Dehydro-TXB\textsubscript{2} (ng/g creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/60</td>
<td>(Cl)</td>
<td>—</td>
<td>788</td>
</tr>
<tr>
<td>2/M/66</td>
<td>(Cl), HT</td>
<td>+</td>
<td>1196</td>
</tr>
<tr>
<td>3/F/77</td>
<td>(Cl), h-Ch</td>
<td>—</td>
<td>2160</td>
</tr>
<tr>
<td>4/M/49</td>
<td>dizziness</td>
<td>+</td>
<td>820</td>
</tr>
<tr>
<td>5/F/70</td>
<td>Cl, DM, HT, h-Ch</td>
<td>—</td>
<td>1304</td>
</tr>
<tr>
<td>6/M/63</td>
<td>DM, HT</td>
<td>+</td>
<td>1628</td>
</tr>
<tr>
<td>7/M/63</td>
<td>RIND, HT</td>
<td>—</td>
<td>1024</td>
</tr>
<tr>
<td>8/M/43</td>
<td>Cl, h-Ch</td>
<td>+</td>
<td>1400</td>
</tr>
<tr>
<td>9/F/74</td>
<td>Cl, HT</td>
<td>—</td>
<td>1212</td>
</tr>
<tr>
<td>10/M/35</td>
<td>Cl</td>
<td>+</td>
<td>1180</td>
</tr>
<tr>
<td>11/M/63</td>
<td>Cl, h-Ch</td>
<td>+</td>
<td>3760</td>
</tr>
<tr>
<td>12/M/67</td>
<td>Cl</td>
<td>—</td>
<td>2152</td>
</tr>
<tr>
<td>13/F/72</td>
<td>Cl</td>
<td>—</td>
<td>1220</td>
</tr>
<tr>
<td>14/M/46</td>
<td>Cl</td>
<td>—</td>
<td>732</td>
</tr>
<tr>
<td>15/M/72</td>
<td>Cl</td>
<td>—</td>
<td>1012</td>
</tr>
<tr>
<td>16/M/44</td>
<td>Cl</td>
<td>—</td>
<td>760</td>
</tr>
<tr>
<td>17/M/50</td>
<td>Cl, HT</td>
<td>+</td>
<td>922</td>
</tr>
<tr>
<td>18/M/62</td>
<td>Cl, DM, h-Ch</td>
<td>+</td>
<td>1572</td>
</tr>
<tr>
<td>19/M/69</td>
<td>Af</td>
<td>—</td>
<td>1052</td>
</tr>
<tr>
<td>20/M/77</td>
<td>Cl, HT</td>
<td>+</td>
<td>2860</td>
</tr>
<tr>
<td>21/M/53</td>
<td>Cl, HT</td>
<td>+</td>
<td>1101</td>
</tr>
<tr>
<td>22/M/80</td>
<td>HT</td>
<td>—</td>
<td>1780</td>
</tr>
<tr>
<td>23/M/71</td>
<td>(Cl), MI, HT</td>
<td>+</td>
<td>1704</td>
</tr>
<tr>
<td>24/M/44</td>
<td>HT</td>
<td>+</td>
<td>1108</td>
</tr>
</tbody>
</table>

F, female; M, male; Cl, cerebral infarction; (Cl), asymptomatic cerebral infarction; DM, diabetes mellitus; HT, hypertension; TIA, transient ischemic attack; RIND, reversible ischemic neurological deficit; Af, atrial fibrillation; MI, myocardial infarction; h-Ch, hypercholesteremia.

are summarized in Table I.

High-resolution real-time B-mode ultrasonography was performed with a 7.5 MHz transducer (SSD-650, Aloka, Tokyo, Japan) to determine the severity of carotid atherosclerosis in 24 patients. The severity of carotid atherosclerosis in each subject was evaluated by plaque score, which was computed by summing the maximum thickness of plaque measured in millimeters on the near and far walls at each of four divisions of both sides of the carotid arteries according to the method of Honda et al.\textsuperscript{12} The clinical data for the 24 patients in whom the plaque score of the extracranial carotid artery was measured are summarized in Table II.

Patients were considered hypertensive if they were taking antihypertensive agents and/or if their blood pressure measured in the hospital was >160 mmHg systolic and/or >95 mmHg diastolic. Patients were considered diabetic if they required treatment with oral hypoglycemic agents or insulin to maintain their level of glycated hemoglobin (HbA\textsubscript{1c}) below 7.0%. Patients were considered hypercholesteremic if they were taking antihypercholesteremic agents and/or if their serum cholesterol level exceeded 240 mg/dl. None of the patients had received drugs known to interfere with prostaglandin metabolism.

In a preliminary experiment to determine the daily profile, the deviation in urinary 11-dehydro-TXB\textsubscript{2} excretion, which was measured in single-voiding urines from the same subject and expressed as ng/g creatinine, was less than 10% between 0000 and 1200. Therefore, urine samples were collected by single-voiding from 0900 to 1100, and frozen immediately at $-20^\circ\text{C}$ until assayed. Urinary 11-dehydro-TXB\textsubscript{2} was measured by radioimmunoassay using monoclonal anti-
body as described previously. Informed consent was obtained from each subject.

Data are expressed as the mean values ± SD. Statistical analysis was performed using Bonferroni’s modified t-test to examine differences in 11-dehydro-TXB₂ values among the groups. A p value of <0.05 was considered significant. Multivariate linear regression analysis was used to assess the correlation with 11-dehydro-TXB₂ for plaque score, age, smoking, hypercholesteremia, diabetes mellitus, sex and hypertension, and was carried out using the HALBAU statistical package on a personal computer (PC-9801 NEC, Tokyo, Japan).

RESULTS

As shown in Fig. 1, urinary excretion of 11-dehydro-TXB₂ in healthy subjects was higher (P<0.01) in cigarette smokers (1063 ±244 ng/g creatinine) than in non-smokers (815 ±183 ng/g creatinine). Aspirin significantly suppressed 11-dehydro-TXB₂ excretion (266 ±114 ng/g creatinine). Among the healthy non-smokers and non-heavy-alcohol drinkers, significant positive correlations were found between urinary 11-dehydro-TXB₂ excretion and total cholesterol, LDL-cholesterol, and atherogenic index (Fig. 2). Sex, age, and body mass index were not significantly associated with 11-dehydro-TXB₂ excretion in healthy non-smokers.

Urinary excretion of 11-dehydro-TXB₂ in the 24 patients who were examined by ultrasonography was 1435±713 ng/g creatinine, and there was no significant difference in 11-dehydro-TXB₂ excretion between patients with or without cerebral infarction. There was a significant positive linear correlation between urinary 11-dehydro-TXB₂ excretion and plaque score in these 24 patients (Fig. 3). Multiple regression analysis of uri-

---

Fig. 1. Urinary 11-dehydro-TXB₂ excretion in healthy subjects (non-smokers, smokers) and patients under chronic aspirin treatment.

Fig. 2. Correlation between urinary 11-dehydro-TXB₂ excretion and cholesterol metabolism: (a) total cholesterol, (b) LDL-cholesterol, and (c) atherogenic index.
nary 11-dehydro-TXB$_2$ excretion and risk factors for cerebral infarction was performed. Multivariate analysis indicated a significant positive correlation between urinary 11-dehydro-TXB$_2$ excretion and hypercholesteremia, age, plaque score and smoking, and a significant negative correlation between urinary 11-dehydro-TXB$_2$ excretion and diabetes mellitus (Table III).

DISCUSSION

Urinary excretion of 11-dehydro-TXB$_2$ is higher than that of 2,3-dinor-TXB$_2$. Thus, 11-dehydro-TXB$_2$ appears at present to be the best index metabolite of systemic TXA$_2$ activity.$^{3,16}$ The value of urinary 11-dehydro-TXB$_2$ excretion, which we determined by radioimmunoassay, was $815 \pm 183$ ng/g crea-
atine in healthy non-smokers, which was quite close to the GC/MS values given in other reports.\textsuperscript{4,9} Chronic aspirin treatment significantly suppressed urinary 11-dehydro-TXB\textsubscript{2} excretion. Catella and Fitzgerald also reported that aspirin caused a substantial suppression of urinary 11-dehydro-TXB\textsubscript{2} (82±4.9%).\textsuperscript{4}

Among healthy subjects, the urinary excretion of 11-dehydro-TXB\textsubscript{2} was higher in cigarette smokers than in non-smokers. These results indicate that cigarette-smoking activates platelets in vivo. Whole smoke, nicotine, and carbon monoxide have been shown to damage endothelium and to promote atherosclerosis. Smoking two cigarettes has been shown to increase circulating endothelial cells by 50% in a human study.\textsuperscript{17} In addition, smokers have been reported to exhibit alterations in platelet aggregation and survival that produce thrombosis.\textsuperscript{18} Lorenz et al. also reported an increased production of endogenous TXA\textsubscript{2} in smokers.\textsuperscript{19} This increased production of TXA\textsubscript{2} may be consistent with a report that the duration of cigarette smoking is the most significant predictor of the presence of internal carotid artery atherosclerosis.\textsuperscript{20}

Low HDL-cholesterol and elevated LDL-cholesterol have both been associated with coronary heart disease.\textsuperscript{21} Our finding of an increase in urinary 11-dehydro-TXB\textsubscript{2} excretion with total cholesterol, LDL-cholesterol and atherogenic index suggests that cholesterol abnormalities may cause atherosclerosis, which could lead to platelet activation. Davi et al. have also reported an increased production of endogenous TXA\textsubscript{2} in type IIa hypercholesterolemia.\textsuperscript{22}

The plaque score was not measured in the 44 healthy subjects. Therefore, it was impossible to investigate the effect of carotid atherosclerosis on urinary 11-dehydro-TXB\textsubscript{2} excretion in the healthy subjects.

In the present examination of the 24 patients in whom the plaque score was measured, hypercholesteremia, age, plaque score and smoking showed a significant positive correlation with 11-dehydro-TXB\textsubscript{2} excretion. The correlations with hypercholesteremia and smoking are in good accord with those obtained in healthy subjects. On the other hand, we did not observe a significant correlation with age in healthy subjects. The plaque score-measured patients were older than the healthy subjects, which may have produced the difference between these two groups. Further investigation is required to clarify the relationship between age and platelet activation.

Diabetes mellitus showed a significant negative correlation with 11-dehydro-TXB\textsubscript{2} excretion. In contrast, Type II diabetes increases has been reported to increase 11-dehydro-TXB\textsubscript{2} excretion and activate platelets in vivo.\textsuperscript{23} The reason for the discrepancy between these results is still uncertain. Davi et al.\textsuperscript{23} studied patients with Type II diabetes who had macrovascular disease. Thus, diabetes may decrease 11-dehydro-TXB\textsubscript{2} excretion if it is evaluated as an independent variable. Further studies are needed to ascertain this possibility. Although hypertension is undoubtedly the most important factor in carotid atherosclerosis,\textsuperscript{24} we failed to confirm the relation between hypertension and urinary 11-dehydro-TXB\textsubscript{2}. Our patients with hypertension were taking antihypertensive agents, which makes evaluation of its influence difficult. There are other quantitative indices of platelet activation, such as plasma levels of β-thromboglobulin and platelet factor 4. Therefore, further studies using various indices may be able to clarify this issue.

In conclusion, our study demonstrated that risk factors for atherosclerosis, such as age, hypercholesteremia, and smoking, activate platelets in vivo and patients may develop carotid atherosclerosis. Moreover, carotid atherosclerosis per se activates platelets and these factors may lead to a vicious cycle which produces atherosclerosis.

Acknowledgments

We wish to express our thanks to Prof. Shozo Yamamoto and Dr. Yoko Hayashi for their generous gift of antiserum to 11-dehydro-TXB\textsubscript{2} and to Ono Pharmaceutical Co. for the generous gift of 11-dehydro-TXB\textsubscript{2} standard.

REFERENCES

1. HAMBERG M, SVENSSON J, SAMUELSSON B: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc Natl Acad Sci USA 1975; 72: 2994–2998
2. FITZGERALD DJ, ROY L, CATELLA F, FITZGERALD GA: Platelet activation in unstable

Japanese Circulation Journal Vol.58, June 1994


15. YANAI H, TAKAGI H (Eds.): Handbook of Multivariate Analysis (in Japanese), Kyoto, Gendai-Sugaku-Sha, 1989; 18–69


