Usefulness of the Measurement of Plasma \( \beta \)-Thromboglobulin (\( \beta \)-TG) in Cerebrovascular Disease

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SUMMARY The plasma concentration of the platelet-specific protein \( \beta \)-thromboglobulin (\( \beta \)-TG) was measured in 39 normal subjects and 568 patients of neurological diseases. The \( \beta \)-TG RIA commercially available KIT was also evaluated. Abnormally high plasma levels of \( \beta \)-TG were demonstrated in groups of ischemic or obstructive cerebrovascular diseases as compared with that of normal subjects.

The highest concentrations were found in 8 patients with Moya-Moya disease, (mean concentration of \( \beta \)-TG was 204.4 ng/ml), completed stroke at an acute stage was next (mean \( \beta \)-TG level was 194.8 ± 70.8 ng/ml). On the other hand, many hemorrhagic cerebro-vascular diseases or other neurological diseases such as brain tumors, hydrocephalus, etc. do not show elevated \( \beta \)-TG levels.

In many patients with ischemic or obstructive cerebro-vascular diseases treated with anti-platelet drugs such as Aspirin, Dipyridamole, Bencyclane or Ticlopidine, a significant fall in plasma concentration of \( \beta \)-TG was chronologically demonstrated.

The measurement of plasma \( \beta \)-TG concentration may be useful not only in the diagnosis of ischemic or obstructive cerebro-vascular disorders but also in judging the efficacy of anti-platelet therapies and prognosis.

A PLATELET SPECIFIC PROTEIN, isolated and termed \( \beta \)-thromboglobulin (\( \beta \)-TG) by Moore et al, has a molecular weight of 3600 which is thought to be composed of 6 identical subunits and is probably located in the \( \alpha \)-granules of blood platelets. The function of \( \beta \)-TG is not yet clear, but it is possibly a “granule packing protein” stabilizing the active constituents in the \( \alpha \)-granules.

Platelet aggregation is associated with the release of a number of proteins, including this platelet specific \( \beta \)-globulin. Plasma levels of \( \beta \)-TG, therefore, reflect platelet aggregation in vivo that is raised on the platelet release reaction.

Recently radioimmunoassays, which would enable the platelet release reaction to be monitored in vivo by measuring the plasma concentration of \( \beta \)-TG and platelet factor 4, have been developed.

Since July 1978, we have measured \( \beta \)-TG by \( \beta \)-TG RIA Kit in many cases of neurosurgical diseases, especially in cerebrovascular disorders.

This report presents the results of a study of neurosurgical patients in whom \( \beta \)-TG has been chronologically measured and examines the availability of measurement of \( \beta \)-TG in ischemic cerebrovascular diseases.

Materials and Methods

Patients

From July 1978 to June 1982, \( \beta \)-TG was measured in 607 patients including 39 normal subjects at Kobe University and Nishiwaki Hospitals.

There were 340 men and 267 women aged 5 to 78 years (mean age 51) of whom 150 had completed stroke; 117 had TIA or RIND, 136 cerebral arteriosclerosis, 43 hypertensive intracerebral hematoma, 19 subarachnoid hemorrhage, 17 chronic subdural hematoma, 5 disseminated intravascular coagulopathy (DIC), 81 other neurological diseases and 39 were normal subjects. Brain tumors, head injuries, hydrocephalus, Parkinsons’ diseases and some degenerative diseases of CNS were included in other neurological diseases (table 1).

Among these cases, 233 were neuro-radiologically studied by angiography and CT (table 2). The plasma \( \beta \)-TG values were determined on a total of 1063 occasions and repeatedly measured in many ischemic or obstructive cerebrovascular diseases. The change of \( \beta \)-TG values were chronologically examined by repeated measurement in 63 cases of ischemic or obstructive diseases with or without anti-platelet therapy using Aspirin, Bencyclane, Dipyridamole, Ifenprodil tartrate or Ticlopidine.

Blood Sampling

Venous blood (2.5ml) was obtained from an antecubital vein or femoral vein using 21-gauge needle. Great care was taken to perform a nontraumatic venipuncture using minimal or no venous occlusion. As Ludlam and Cash recommended, sample preparation is the critical stage in this assay. Each sampling was obtained by a skilled technician.

Venous blood was collected into a 3-ml plastic syringe and immediately transferred into a precooled plastic tube with 0.3ml anticoagulant mixture containing EDTA and theophylline.

Within 2 minutes of collection of blood, each tube was gently inverted 2 or 3 times and was quickly placed in an ice water bath for 15 minutes. Platelet-poor plasma was prepared by centrifugation at 2000 g for 30 minutes at 2–4°C and the top 0.5 of platelet-poor plasma was carefully collected and stored at −20°C for \( \beta \)-TG assay.
The plasma concentrations of β-TG were measured by radioimmunoassay. The β-TG radioimmunoassay (β-TG RIA Kit) was purchased from the Radiochemical Centre (KAKEN Chemical Co.).

The radioactivity was measured by Aloka auto well gamma system ARC-351, and processed by Aloka ACM-702.

**Results**

1. **Basic Investigation of β-TG RIA Kit**

The influence of incubation temperature among 4°C, 25°C and 37°C on the standard curves of β-TG was negligible.

When platelet-poor plasma, after centrifugation, was divided into 3 different portions (upper, middle and lower), no difference on β-TG values was noticed. (The coefficient of correlation was 0.9992 among the 3 portions.) In addition, coefficient of variation on intraassay and interassay reproducibility of β-TG values, as well as the mean percentage recovery, were quite satisfactory. From the results of these basic investigations, the β-TG RIA Kit was thought to be stable (fig. 1).

2. **Clinical Studies**

1. **Normal Subjects**

In order to establish a normal mean and range for our own series, 41 random blood samples were taken from 39 healthy volunteers (19 male and 20 female), mean age 28.7 years (range 18–54). No subject was taking any drug known to affect platelet function *in vivo*. The mean concentration of β-TG in the 39 normal subjects was 22.3 ± 12.0 ng/ml (SD) (range 6.0–49.2 ng/ml). The mean value of β-TG among the males was not significantly different from that of females. But when related to age, the concentration rose to some degree as the age increased. There was no correlation between plasma β-TG and platelet count.

2. **Cerebrovascular Diseases**

Among 70 patients with recently completed stroke (acute stage), a markedly raised plasma β-TG level, with a mean value of 194.8 ± 70.8(SD) ng/ml, was found in almost all cases. In contrast, the plasma β-TG level in 80 stroke patients in the chronic stage, the mean concentration being 85.8 ± 63.3(SD) ng/ml, was not so elevated as that of acute stage. β-TG, estimated in the plasma of 117 patients with TIA or RIND, revealed a mean value of 121.3 ± 79.2(SD) ng/ml. This was also significantly increased compared to values obtained in samples from normal subjects (fig. 2).

In addition, 136 patients, who were diagnosed with so-called cerebral arteriosclerosis, have revealed a slightly high plasma β-TG level with a mean level of 73.5 ± 57.3(SD) ng/ml.

On the other hand, the normal range of plasma β-TG concentration was obtained in almost all hemorrhagic cerebrovascular diseases. There were patients with hypertensive intracerebral hematoma and subarachnoidal hemorrhage, with a mean β-TG level of 34.3 ± 20.7(SD) ng/ml and 51.5 ± 41.5(SD) ng/ml respectively.
Plasma β-TG Concentration in various neurological disease

<table>
<thead>
<tr>
<th>Condition</th>
<th>β-TG Concentration (ng/ml)</th>
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</thead>
<tbody>
<tr>
<td>Normal adult (control)</td>
<td></td>
</tr>
<tr>
<td>Completed stroke</td>
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<tr>
<td>Acute stage</td>
<td></td>
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<tr>
<td>Chronic stage</td>
<td></td>
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<tr>
<td>T.I.A. &amp; RIND</td>
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<tr>
<td>Cerebral arteriosclerosis</td>
<td></td>
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<tr>
<td>Intracerebral hematoma</td>
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<tr>
<td>SAH</td>
<td></td>
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<tr>
<td>Chronic subdural hematoma</td>
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</tr>
<tr>
<td>Other neurological diseases</td>
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</tbody>
</table>

However, a β-TG plasma level in the patients with chronic subdural hematoma had a wide range from 11 to 250 ng/ml.

Other neurological diseases included brain tumors, hydrocephalus, Parkinson’s disease and so on. Almost all patients in this group showed a normal plasma concentration of β-TG with a mean level of 34.7 ± 15.4 (SD) ng/ml (fig. 2).

Among 233 patients who were neuro-radiologically confirmed, a mean plasma concentration of β-TG in 49 patients of recent cerebrovascular occlusion was 135.8 ng/ml. In 8 Moya-Moya diseases, the highest β-TG concentration mean level of 204.4 ng/ml has been obtained. However, the plasma β-TG level in 25 patients with cerebrovascular stenosis and in patients with cerebral arteriosclerosis were 100 ng/ml and 77.7 ng/ml respectively. These levels were lower than those of complete cerebrovascular occlusion (fig. 3).

These ischemic cerebrovascular disease patients were treated with antiplatelet drugs such as Aspirin, Dipyridamole, Ifenprodil tartrate and Ticlopidine. Plasma β-TG level decreased remarkably in 24 patients with obstructive cerebrovascular disease who were measured more than 2 times and were followed over one month.

Among high plasma β-TG level stroke patients, β-TG concentration returned to normal in about two thirds of the patients upon treatment with Aspirin or Ifenprodil tartrate. In contrast, the β-TG levels of 4 untreated cases remained at a high level (fig. 4).

Neuroradiological Diagnosis of CVA (Angiography & CT)

<table>
<thead>
<tr>
<th>Condition</th>
<th>β-TG Concentration (ng/ml)</th>
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<tr>
<td>Cerebrovascular obstruction</td>
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<tr>
<td>Moya-Moya disease</td>
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<tr>
<td>Cerebrovascular stenosis</td>
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<tr>
<td>Cerebral arteriosclerosis</td>
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<tr>
<td>Intracerebral hematoma</td>
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<tr>
<td>Aneurysm or AVM</td>
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</table>

Figure 2. Plasma concentration of β-TG in various neurological diseases and normal subjects.

Figure 3. Plasma concentration of β-TG in cerebrovascular diseases confirmed by angiography and CT.
Chronological change of β-TG concentration in obstructive cerebrovascular diseases

A total of 85 measurements was periodically taken during 15 months. The concentration of β-TG had fallen to normal level in about one third of 23 patients who had been treated with Aspirin, Dipyridamole, Ifenprodil tartrate and Ticlopidine. Four cases untreated with anti-platelet drugs had still high β-TG concentration in each 15 measurements.

Figure 5 and figure 6 describe the chronological change of β-TG concentration in 35 patients with TIA or RIND. Plasma β-TG levels in 24 patients who had been treated with antiplatelet drugs fell to near normal range. But β-TG concentration in untreated patients or patients who stopped the treatment on the way, remained at a high level or raised gradually.

As far as we have examined, there was no significant difference in the value of β-TG between Aspirin and Ticlopidine treated groups, but these drugs were more effective than Dipyridamole or Ifenprodil tartrate from a quantitative point of view.

**Case Report**

The plasma concentration of β-TG was repeatedly measured in a 47-year-old woman with Moya-Moya disease every 2–4 days during 6 weeks and every 1–2 months after that. This woman had a history of 4 TIAs during the previous 6 months and finally left mild hemiparesis and hemihypesthesia when she was admitted in our clinic. Bilateral CAG was performed which demonstrated the obstruction of her right internal carotid artery at the CI portion and abnormal small collateral vessels, called “Moya-Moya vessels,” in its distal portion. Her plasma level of β-TG was 162.5 ng/ml upon admission. In spite of the treatment with Urokinase and low molecular dextran for two weeks, neither clinical symptoms nor the level of β-TG improved. Then, following an operation of STA-MC anastomosis her left hemiparesis and hypesthesia improved. But her plasma concentration of β-TG was still abnormally high. The β-TG concentration fell by the administration of Aspirin (1.5 g/day, p.o.) for 10 days after operation. Her antiplatelet drug was changed to Bencyclane (300 mg/day, p.o.) because of gastro-intestinal trouble. As the plasma level of β-TG rose further, again, Aspirin (1.2 g/day, p.o.) was administered and its dosage was gradually reduced dur-
ing one year. No TIA occurred in spite of a somewhat abnormal level of β-TG (fig. 7).

Discussion

Ischemic or obstructive cerebrovascular diseases occupy about 70% of all cerebrovascular diseases, which, in turn, are the foremost cause of death in our country. The diagnosis of CVA has been remarkably developed by CT in addition to angiography.

However, the diagnosis of microthrombus is not always able to be diagnosed by new neurological methods, especially the location of the microthrombus. On the other hand, another adjunctive diagnostic method to discover the formation of thrombus is to examine the function of platelet aggregation and release. Platelet aggregation and release and fibrin formation are thought to occur in thromboembolism, arterial disease, and intravascular coagulation.

Recently, increased numbers of circulating platelet aggregates have been found in patients with TIA.\textsuperscript{15, 33} β-Thromboglobulin (β-TG), Platelet factor 4 (PF 4),\textsuperscript{14} Platelet derived growth factor (PDGF),\textsuperscript{45} and Thrombin sensitive protein (TSP)\textsuperscript{4} are platelet-specific proteins that are contained in the α-granule of the platelet.

The molecule of β-TG is composed of six identical subunit polypeptides, each with a molecular weight of 6000,\textsuperscript{32} and a biological half-life of about 100 minutes is observed.\textsuperscript{12, 36}

Recently, there have been many reports suggesting that increased plasma levels of β-TG or PF4 may serve as an aid in the diagnosis\textsuperscript{8} or confirmation of certain diseases stated known or suspected to be associated with platelet activation or destruction.\textsuperscript{7, 23}

The mean level of β-TG in plasma of normal subjects, which has been reported so far as 30.5 ± 12.8 ng/ml by Ludlam,\textsuperscript{26} 29.8 ng/ml by Denham,\textsuperscript{13} 24.9 ng/ml by Burrow,\textsuperscript{9} 34 ± 20 ng/ml in healthy Japanese aged over 60 years by Matsuda,\textsuperscript{29} 28.9 ng/ml in male, 26.1 ng/ml in female by Cella,\textsuperscript{11} 17.8 ng/ml by Kaplan\textsuperscript{23} and 22.3 ± 12.5 ng/ml by us.

Raised levels of β-TG have been reported in diabetes mellitus\textsuperscript{10} and venous thrombosis,\textsuperscript{11, 13, 25, 42} According to Denhams' report, plasma β-TG level of mural thrombosis in acute myocardial infarction was 108.6 ng/ml and he has suggested that a β-TG concentration greater than 82 ng/ml is indicative of thrombosis.\textsuperscript{13}

Preston has also reported the raised levels of β-TG in diabetes mellitus with microangiopathy and empha-
sized the usefulness of measurement of $\beta$-TG.38

Redman has reported that pre-eclampsia, which was thought to have abnormal activation of the coagulation system, had significantly higher $\beta$-TG levels than normal pregnant patients.40

There are some reports of raised $\beta$-TG level in disseminated intravascular coagulopathy (DIC) 25 as in our data, but the mechanism of raised $\beta$-TG level has not been proved.

There are few reports of the plasma $\beta$-TG concentration in cerebrovascular diseases.37,46

According to the report by Pepper,37 raised $\beta$-TG levels were noticed in patients with recent cerebrovascular accidents (less than 4 days old) which might have been due to thrombus formation in cerebral arteries. On the other hand, that of old CVA (greater than 30 days) was normal. However, because the case size in this report of CVA was small, the statistically significant difference was unclear. Yamamoto46 has reported that mean $\beta$-TG levels in the acute stage of cerebral thrombosis (within 30 days) were significantly elevated, with a plasma $\beta$-TG level over 100 ng/ml in 21% of the patients. Maruyama28 has also reported that the mean concentration of $\beta$-TG in TIA patients was 113 ng/ml and that of $\beta$-TG in cerebral thrombotic patients in the acute stage was 117.3 ng/ml. In the chronic stage of thrombosis, the value of $\beta$-TG is believed to become gradually normal. However, in our series, raised $\beta$-TG levels were still detected in some of these stroke or TIA patients in the chronic stage.

According to the report of Farrell,18 plasma $\beta$-TG levels were significantly raised in patients with advanced malignancy when compared with control, but no raised $\beta$-TG levels were noticed in patients with malignant brain tumors except for the patients with DIC as far as we have examined.

Nonsurgical treatment for cerebral thrombosis is subdivided into three groups which include thrombolytic therapy, anticoagulant therapy, and antiplatelet therapy. So far, thrombolytic therapy with Urokinase and anticoagulant therapy with Heparin or Warfarin were mainly used for thromboembolic diseases. But recently the study of the role of platelets for thrombosis and antiplatelet therapy have developed rapidly. Its achievements have become a center of attraction.20,27,31,34,44

Aspirin,16,19,22 Dipyridamole,1,21,41 Sulfinpyrazone,4,6,17 Clofibrate,2 Bencyclane fumarate24 and Ticlopidine43 are generally used as antiplatelet drugs. Among these drugs, it has been proved that Aspirin has the effect of prevention of TIA in the double blind test by Field et al.,19 and other authors.16,22 There are inconsistent results in stroke patients treated with Dipyridamole, one is effective,39 another is not effective.1

Dougherty15 has reported that circulating platelet aggregates were elevated in patients with TIA, but Aspirin and Dipyridamole failed to block the formation of platelet aggregates in acute cerebral ischemia. So, platelet activation was abnormal in acute cerebral ischemia but usually returned to normal with or without anti-platelet therapy. It has been observed that Sulfinpyrazone was effective in the patients with stroke,6 and Amaurosis fugax.5,17

Recently, Ticlopidine has been reported to be a potent, long-lasting inhibitor of platelet aggregation and to exert a potent anti-thrombotic action.3 Clinically, there is only one report of the treatment of a patient with TIA and RIND with Ticlopidine (by Uchiyama) which has been proved to be effective.43 We have also recognized not only the preventative effect of Ticlopidine for TIA or RIND, but also its normalization of plasma $\beta$-TG level.

There are only a few reports about the change of plasma $\beta$-TG levels during the treatment with antiplatelet drugs. Matsuda46 has reported that a statistically significant decrease in the levels of $\beta$-TG in plasma was observed after Dipyridamole was given to thromboembolic patients. Our data also indicates that Aspirin and Ticlopidine may have desirable effects in many cases of ischemic disease. We have an impression that Ticlopidine is more advantageous than Aspirin because the latter has some unfavorable side effects such as gastro-intestinal trouble and prolonged bleeding time59 if it is used for long time.

As it has already been mentioned by many authors, the measurement of plasma $\beta$-TG level is one of the useful supportive methods which is able to show the functional dynamics of the platelet in vivo. However, one should prepare the sample for the measurement of plasma $\beta$-TG as described above, and with the greatest care in order to get the exact value.

The measurement of plasma $\beta$-TG level seems to be not only one of the useful new methods to diagnose thrombotic cerebrovascular disease but also a supplementary method to determine prognosis and the therapeutic effect of any antiplatelet drug used. However, much basic research about definite function associated with $\beta$-TG should be the subject for future study, because it is still unknown whether hypercoagulability predisposes thrombus formation or whether the presence of thrombus causes activation of circulating platelets. Any final conclusion about the efficacy of anti-platelet drugs should be decided from clinical observation in prospective controlled studies.

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