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Plasma β-Thromboglobulin Levels in Chronic Renal Failure Patients

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ABSTRACT

Impaired platelet function is commonly recognized in uremia. It is partially improved by dialysis. However, in regular hemodialysis patients (RD), platelet function is not fully evaluated. This study investigated platelet activity in chronic renal failure (CRF) using β-thromboglobulin (BTG) as a marker. BTG in RD were significantly higher than those in non-dialyzed CRF patients ($P<0.05$), and BTG in non-dialyzed CRF were higher than in normal controls ($P<0.01$). In non-dialyzed CRF, BTG were correlated to serum creatinine value ($P<0.01$). Arteriovenous fistula did not affect BTG. Hemodialysis and direct hemoperfusion with charcoal beads increased BTG but neither peritoneal dialysis nor hemofiltration altered BTG. RD treated over one year showed higher BTG than those treated for less than one year ($P<0.01$). In RD who showed elevated BTG, platelet aggregation and retention rates were depressed and bleeding times were prolonged.

These results indicate that BTG can be used as the marker of impaired platelet function in CRF and of the blood compatibility of artificial kidneys.

Key words: β-thromboglobulin, chronic renal failure, hemodialysis, peritoneal dialysis, hemofiltration, arteriovenous fistula

INTRODUCTION

Impaired platelet function such as depressed platelet aggregation and retention and decreased release of platelet factor 3 has been well recognized in uremia.[1] It is regarded as the cause of hemorrhagic diathesis or prolonged bleeding time of uremia. The pathogenesis of platelet dysfunction is attributed to accumulated metabolites such as urea, guanidinosuccinic acid, methyl-guanidine, phenols, c-AMP or excess PGI_2. Dialysis therapy partially improves the platelet function;[2] however, in regular hemodialysis patients (RD), especially in those treated for a long period, it is not fully evaluated.

β-Thromboglobulin (BTG) is one of the releasing factors of platelets and is secreted from the α-granule of the platelet into serum when the platelet is activated.[3] BTG is composed of four chains of protein and may be degraded in or excreted by the kidney. In chronic renal failure (CRF), several factors can affect the BTG level, including hemodialysis procedures, disturbances in platelet function and the state of renal function. To clarify the effects of these factors, we examined plasma BTG in CRF and RD using various types of artificial kidney.

MATERIALS AND METHODS

Patients

Twenty-five patients on regular hemodialysis treatment and 14 patients with CRF on conservative therapy were studied. In non-dialyzed CRF, serum creatinine concentrations were 2.4–12.6 mg/dl and five patients were treated with peritoneal dialysis after the BTG examination. In these patients, BTG were evaluated before and three days after arteriovenous fistula formation. Eleven dialyzed patients were treated with hollow fiber dialyzers of regenerated cellulose membrane (Cordis Dow Corp., Miami, Fla., U.S.A, or Teijin Corp., Tokyo, Japan), eight were treated with hollow fiber dialyzers of Cuprophane membrane (Terumo Corp., Tokyo, Japan), and six were treated with hollow fiber dialyzers of poly-methyl-methacrylate (PMMA) membrane (Toray Corp., Tokyo, Japan). Six patients in RD group were also treated with hemofiltration. Hemofiltration was performed with a PMMA membrane hemofilter (Toray Corp.) by the post-dilution method. Substitution and ultrafiltration volume were 20 L and 21–22 L/treatment, respectively. The frequency of hemodialysis or hemofiltration was thrice a week and the duration of each treatment was approximately five hours. Direct hemoperfusion was performed on five RD with charcoal columns containing 100 gm of non-coated activated charcoal beads (Sumitomo Corp., Osaka, Japan) for two hours.

Blood samples were collected from RD immediately before the treatment, and 30 minutes, 2 and 5 hours after the start of treatment. In peritoneal dialysis, blood samples were examined before and after

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dialysis. Ultrafiltrates or peritoneal fluids were also collected. In non-dialyzed CRF, blood specimens were taken in the fasting state.

Controls

Twenty-eight normal subjects acted as controls.

β-Thromboglobulin. Blood was drawn through an 18-20 gauge needle into a plastic tube and was immediately transferred into a tube containing 15 mM theophylline and 134 mM EDTA previously placed in ice. Centrifugation, at 4°C and 3000 rpm for 30 minutes, was performed within three hours after blood collection. Platelet-poor plasma taken after centrifugation was then assayed for BTG by radioimmunoassay. For the assay, BTG radioimmunoassay kits (Amersham Corp., Arlington Heights, Illinois U.S.A.) were used.

Other studies of platelet function. Platelet aggregation was measured with Dual Sample Aggregation Meter (Sienco Corp., U.S.A.). As the activating agents, ADP, epinephrine, collagen, and ristocetin were utilized. Platelet retention, platelet counts, and volume were performed with modified Saltzman method (Igaku Shoin Corp., Tokyo, Japan), Coulter Counter and Channelyzer (Coulter Electronic Corp., Hialeah, Florida, U.S.A.). Bleeding time was examined by the Ivy template method.

RESULTS

BTG levels in normal controls, non-dialyzed CRF and RD were 24 ± 12, 84 ± 27 and 118 ± 40 ng/ml, respectively. There were noted significant differences between the three groups (Fig. 1). In non-dialyzed CRF, there was significant correlation between plasma BTG values and serum creatinine concentrations (Fig. 2). To examine the effect of arteriovenous fistula on BTG, we compared BTG before and three days after the formation of the fistula. As shown in Figure 3, there was no difference between the two groups. BTG in the venous blood of the shunt-side arm and opposite arm were 104 ± 32 and 96 ± 29 ng/ml, respectively (NS). These results indicate that the arteriovenous fistula does not affect the BTG levels.

Figure 4 shows the effect of hemodialysis on BTG. Hemodialysis with regenerated cellulose or Cuprophane membrane increased BTG values significantly within the first two hours. On the other hand, hemodialysis with PMMA membrane caused only mild elevation of BTG, which was less than that with regenerated cellulose or Cuprophane membrane ($P<0.05$). Hemoperfusion with activated charcoal beads caused remarkable increase in BTG (Fig. 5), much greater than was seen in hemodialysis therapy ($P<0.005$). Figure 5 also shows the transient increase of BTG in the first 30 minutes of hemofiltration. This change was not significant. BTG in filtrate increased with time as shown in Figure 6. The mean content of BTG in filtrate per treatment was about 240 mg. The sieving coefficient of BTG increased from 0 at the initiation to 0.48 at the end of therapy. After re-filtration of the filtrate with Amicon Diaflo membrane DM-5 (cut-off point: 5,000 daltons; Amicon Corp., Lexington, Mass., U.S.A.), BTG was hardly detected in the re-filtrate. These results might indicate that the molecular weight of BTG detected in filtrate is 5,000–20,000

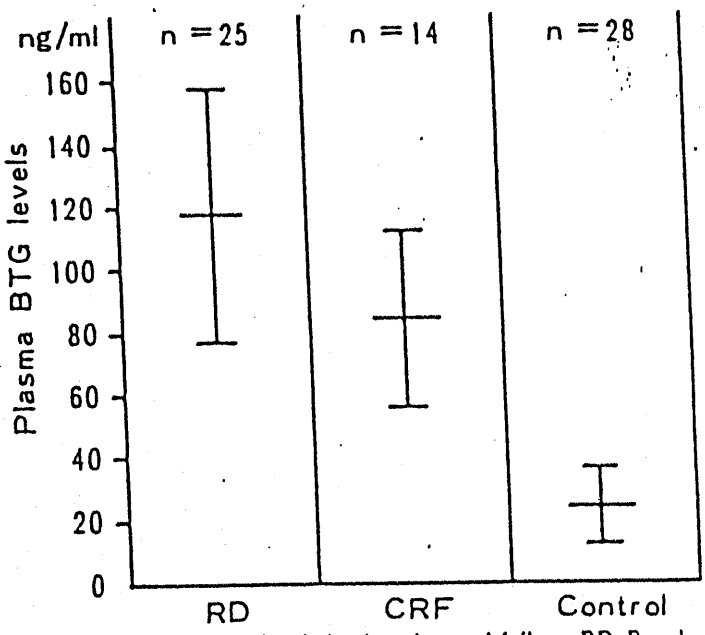

FIG. 1. Plasma BTG levels in chronic renal failure. RD: Regular hemodialysis patients. CRF: Non-dialyzed chronic renal failure patients. RD vs. CRF: $P<0.05$; CRF vs. control: $P<0.01$.

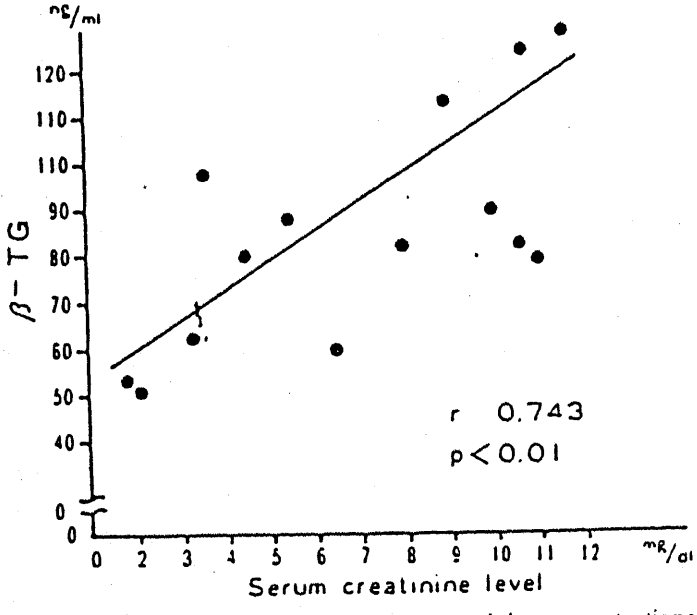

FIG. 2. Plasma BTG levels and serum creatinine concentrations in non-dialyzed chronic renal failure.

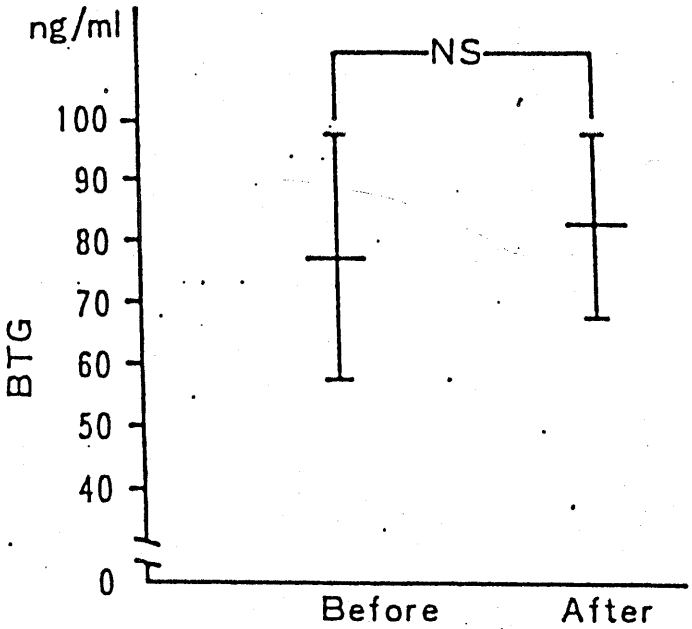

FIG. 3. Plasma BTG levels before and three days after the formation of an arteriovenous fistula.

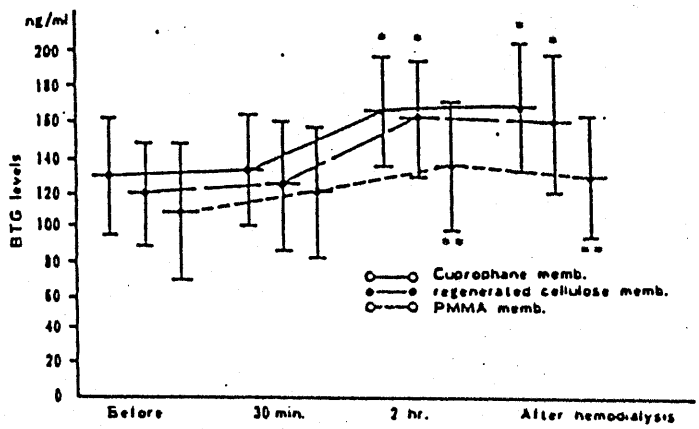

FIG. 4. Plasma BTG levels during hemodialysis. The effect of dialysis membrane on BTG.

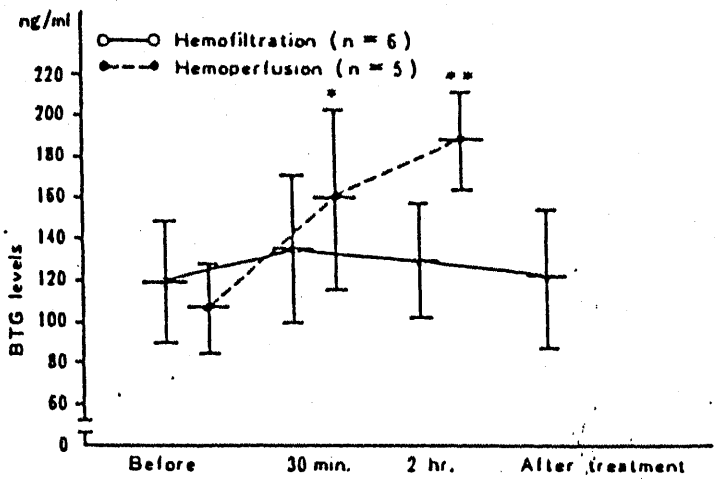

FIG. 5. Plasma BTG levels during hemofiltration and direct hemoperfusion with activated charcoal beads. *P<0.05 vs. the pre-hemoperfusion value. **P<0.005 vs. the pre-hemoperfusion value.

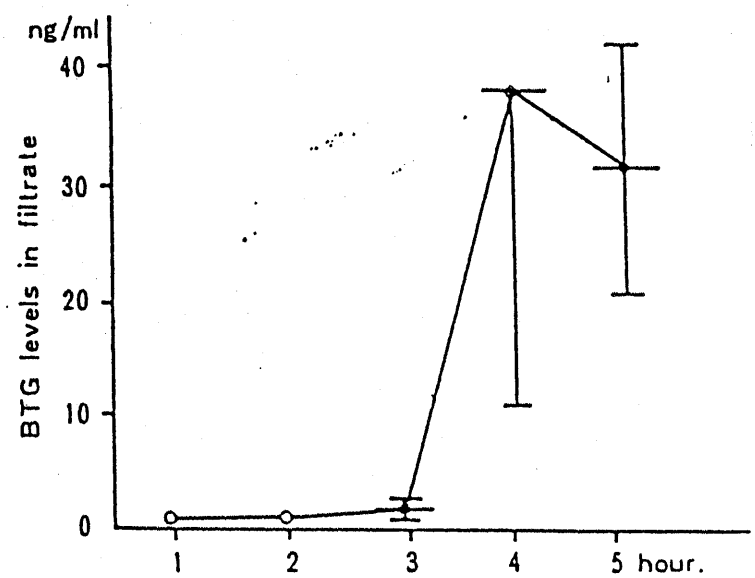

FIG. 6. Filtrate BTG levels during hemofiltration of PL S.Y. (n = 4).

daltons (cut-off point of hemofilter). In peritoneal dialysis, BTG showed moderate but insignificant decrease (Fig. 7). BTG was detected at very low levels in peritoneal dialysis fluids.

To evaluate the long-term effects of hemodialysis on BTG values, we divided RD into two groups, with dialysis periods of less than one year and over one year. As shown in Figure 8, the BTG in the latter was significantly higher than that in the former.

The relationship between BTG and platelet function in RD is shown in Figures 9 & 10. The BTG of patients with normal platelet aggregation was low compared to those with decreased platelet aggregation. Similar relationships were also noted in bleeding time or platelet retention (Fig. 11). The platelet volume did not show any significant relationship to BTG.

DISCUSSION

It has been suggested that elevated BTG level is a valid index of platelet activation or consumption. A high BTG value is recognized in venous thrombosis, myocardial infarction, diabetes mellitus, eclampsia, and other thrombotic diseases. Recently a few papers reported elevated plasma BTG values in CRF.[4-6] Our present results are consistent with these reports, and furthermore, we revealed the correlation between serum creatinine concentrations and BTG values.

The high levels of BTG in CRF could be attributable to increased release of BTG as the consequence of accelerated platelet activation, decreased renal excretion in urine, reduced renal catabolic rate, or a combination of these. It is not likely that high BTG levels in CRF reflect intensified platelet activation because of

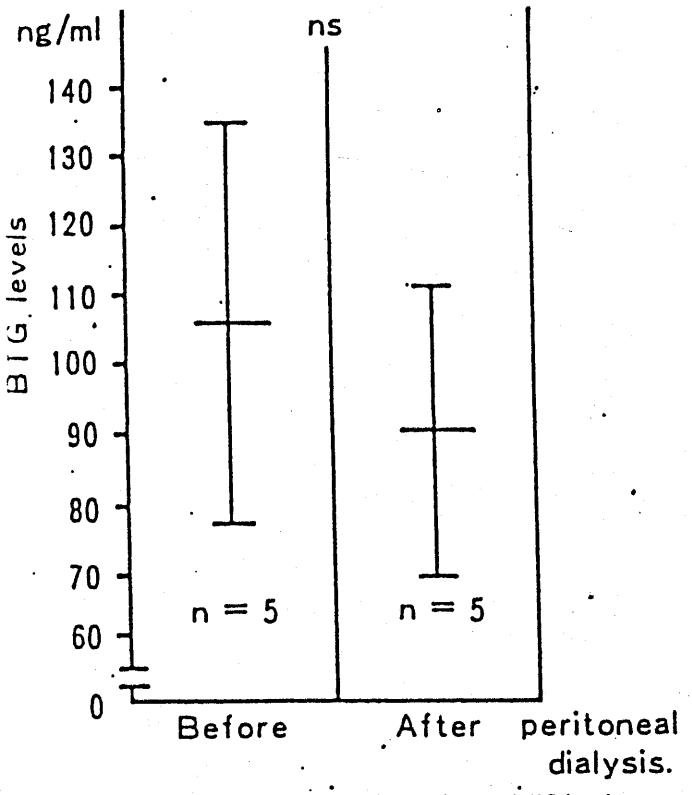

FIG. 7. Effect of peritoneal dialysis on plasma BTG levels.

the correlation between BTG and creatinine. This relationship indicates that high BTG values are due to the retention of BTG in circulating plasma. Urinary BTG levels are undetectable in normal subjects and quite high in nephrotic syndrome, even with impaired renal function.[7] These facts indicate that decrease of urinary excretion of BTG cannot explain the retention of BTG in plasma. Therefore, we conclude that elevated BTG values in plasma are mainly due to decreased renal degradation of this microprotein.

The higher BTG levels in RD compared with non-dialyzed CRF could not be explained only by the progression of impaired renal function. Hemodialysis activates platelets, and causes the increase of BTG. This is clearly shown in Figure 4, and as a consequence, RD treated with hemodialysis for over one year showed higher BTG values than those treated for less than one year. These findings indicate that hemodialysis itself contributes to the elevation of BTG.

We found that the increase of BTG with hemodialysis is affected by the material of the dialysis membrane. The increase of BTG was remarkable with regenerated cellulose and Cuprophane membrane, but was only slight with PMMA membrane. Our previous report revealed that there were no differences between regenerated cellulose and cellulose acetate membrane in increasing the BTG during hemodialysis.[7] On the

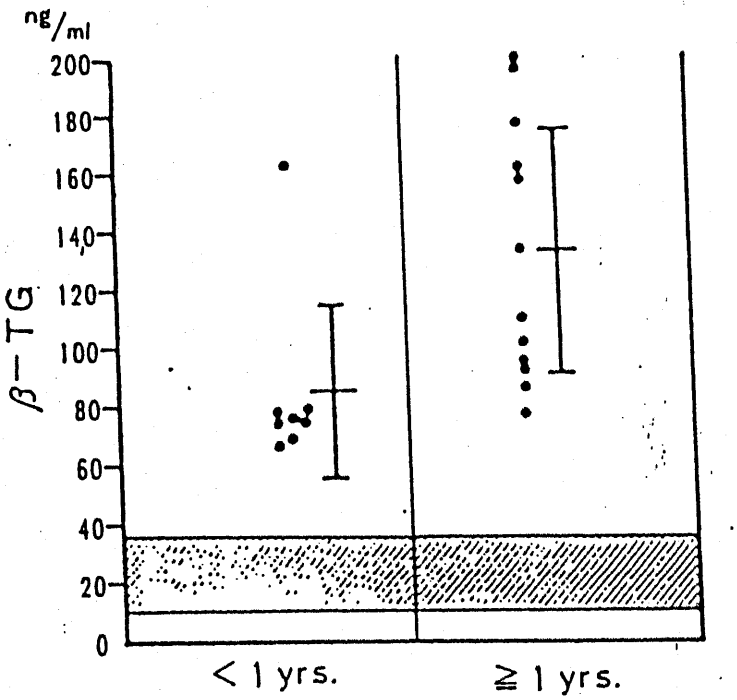

FIG. 8. Effect of hemodialysis period on plasma BTG value ($P<0.01$).

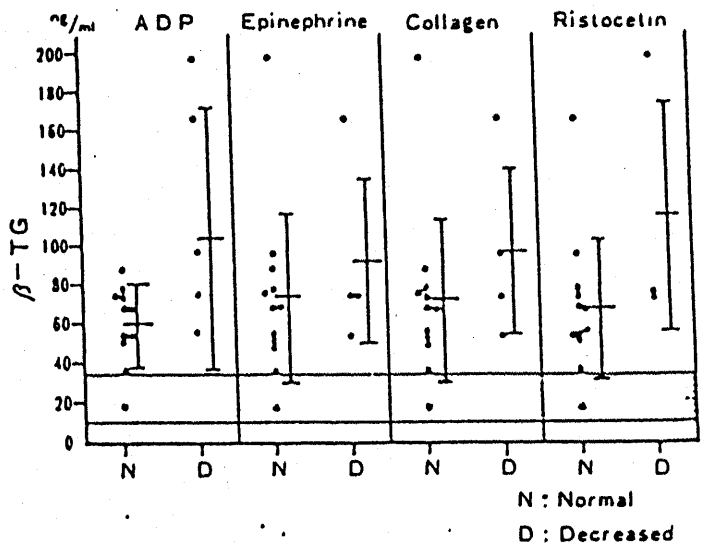

FIG. 9. Platelet aggregation and plasma BTG in regular hemodialysis patients.

other hand, Adler noted a difference in the effect on BTG between regenerated cellulose and Cuprophane membrane.[8]

Hemoperfusion with activated charcoal beads caused remarkable elevations of BTG. It is conceivable that hemoperfusion increased BTG more severely than hemodialysis because the former was less compatible with blood than the latter. It is also explainable in terms of biocompatibility that peritoneal dialysis did not elevate BTG levels.

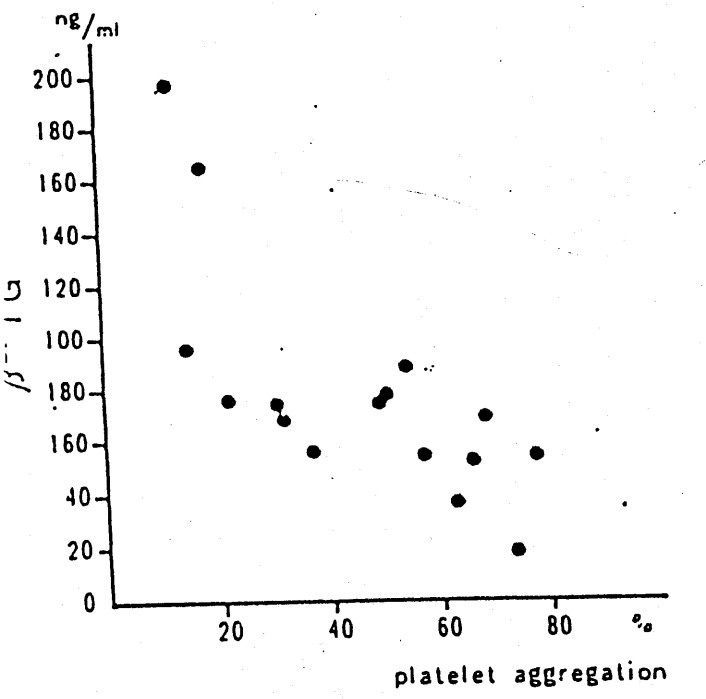

FIG. 10. ADP (10×10^{-4} M)-induced platelet aggregation and plasma BTG.

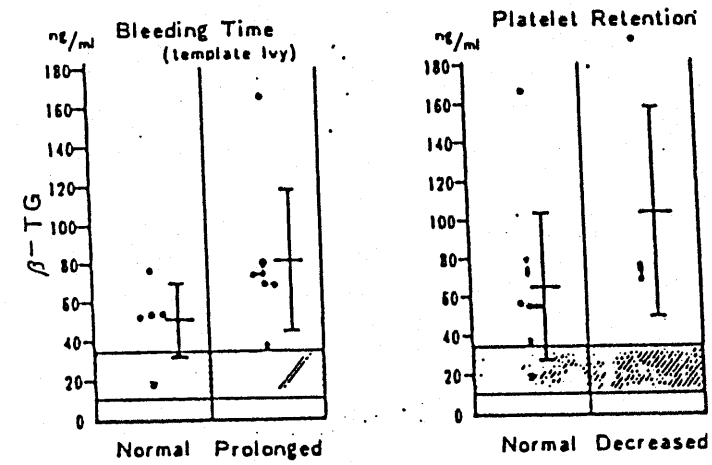

FIG. 11. Relationship between plasma BTG and platelet retention or Ivy bleeding time in regular hemodialysis patients.

In hemofiltration, BTG did not change significantly. This finding could be attributed to adequate blood compatibility (little platelet activation) of the hemofilter, loss of BTG in filtrate, or a combination of both. BTG in filtrate increased gradually during hemofiltration and net BTG excretion in filtrate was about 240 mg/ treatment. The filtered BTG is 5,000–20,000 daltons in molecular weight. BTG consists of four chains of protein; each chain is 8851 in molecular weight.[9] The molecular weight of BTG in its complete form is about 36,000 daltons. This fact suggests that BTG excreted in filtrate is not the complete form of BTG, but is composed of single or double chains of protein. BTG seems to be degraded to single- or double-chained protein composition during hemofiltration, and then passed into the filtrate. As there was no significant change of BTG in the plasma during hemofiltration, this degradation might take place in the hemofilter. This hypothesis explains the delay of the increase of BTG in the filtrate and the slight reduction of BTG in plasma during hemofiltration. The hemofilters used in these experiments were composed of PMMA membrane. As mentioned above, hemodialysis using a PMMA membrane dialyzer caused only a mild elevation of BTG, so stability of BTG in hemofiltration might be partially due to good blood compatibility of the membrane.

BTG was low in RD whose platelet function was within the normal range, and was high in RD whose platelet function was impaired. These findings indicate that continuous activation of platelets (high BTG value) might exhaust the intra-platelet releasing factors, and the exhaustion might depress the platelet function.

In conclusion, high BTG levels in non-dialyzed CRF are mainly due to decreased renal degradation of BTG with the progression of renal failure. In RD, hemodialysis therapy itself accelerates the elevation of BTG. The degree of BTG increase differs according to membrane material and also the mode of treatment. These results indicate that BTG is an excellent marker for the blood compatibility of the artificial kidneys.

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