Detection of Factor VIII Inhibitors With the Partial Thromboplastin Time

By Thomas S. Lossing, Carol K. Kasper, and Donald I. Feinstein

Variations of the partial thromboplastin time (PTT) were tested to determine the best screening method for detection of inhibitors of factor VIII. Variables tested included the duration of preincubation of a mixture of patient plasma and factor VIII source (normal plasma), the ratio of the patient plasma to the normal plasma, and the duration of incubation of the normal plasma–patient plasma mixture with kaolin-cephalin suspension prior to re-calciﬁcation. The following conclusions were reached: (1) The PTT performed on a mixture of equal amounts of patient and normal plasma without preincubation of the mixture was inadequate to detect many factor VIII inhibitors. (2) Factor VIII inhibitors of more than 0.5 Bethesda units could be detected if the PTT was performed on a mixture of four parts patient plasma and one part normal plasma, with preincubation of the mixture for 60 min at 37°C. (3) Factor VIII inhibitors as weak as 0.1 Bethesda units could be detected if the PTT was performed on a mixture of four parts patient plasma and one part normal plasma incubated with kaolin-cephalin suspension for 120 min at 37°C before re-calciﬁcation. The last method may make detection of mild factor VIII inhibitors possible in routine clinical laboratories not equipped to perform the more technically difﬁcult Bethesda inhibitor assays.

ACTIVATED PARTIAL THROMBOPLASTIN TIME (PTT) is the major screening test to evaluate the intrinsic pathway of blood coagulation. A prolonged PTT may be due to a deﬁciency of one or more clotting factors or due to the presence of an inhibitor. To distinguish these conditions, our laboratory usually performs a PTT on a mixture of equal parts of patient and normal plasma incubated for 3 min. The same test is used at the Oxford Haemophilia Center.1 If the patient has a pure clotting factor deﬁciency, the PTT on such a mixture will be nearly normal because of the contribution of the missing clotting factor from normal plasma, whereas in the presence of an inhibitor the PTT on an equal mixture may remain prolonged.

Using this method, we have sometimes failed to detect clinically signiﬁcant mild factor VIII inhibitors arising in patients with classic hemophilia because the PTT on an equal mixture of patient and normal plasma is normal. Therefore a study was undertaken to devise a screening method using the PTT that could detect mild factor VIII inhibitors yet be simple enough for practical use in the routine clinical laboratory.

MATERIALS AND METHODS

Venous blood was drawn into a plastic syringe and mixed with balanced citrate anticoagulant in a ratio of nine parts blood to one part anticoagulant. The blood was centrifuged at 4°C and 12,000 g for 20 min, and the plasma was stored in plastic vials at −30°C before testing. The normal...
PLASMA STANDARD was prepared by pooling fresh plasma from 14 healthy hospital employees who were known to have normal PTTs. The normal standard was also stored at -30°C before use.

The PTT was performed by hand by the method of Proctor and Rapaport. A mixture of patient plasma and normal plasma in a plastic tube was preincubated under various conditions as described below. One part of a suspension of kaolin and human brain cephalin in barbital buffer and one part of the preincubated patient plasma-normal plasma mixture was incubated at 37°C in a glass tube for 3 min and then recalcified. The interval to formation of a clot was recorded in seconds. Each test was performed in duplicate.

Variations of the PTT which were tested included the following:

Duration of preincubation. The patient plasma-normal plasma mixture was preincubated for 0, 15, 20, 60, 90, or 120 min.

Ratio of patient plasma and normal plasma. The ratios used were either one part patient plasma mixed with one part normal plasma or four parts patient plasma mixed with one part normal plasma.

Duration of incubation of the PTT. The kaolin-cephalin suspension was incubated with the patient-normal plasma mixture (not preincubated) in a glass tube at 37°C for 3, 15, 20, 60, 90, or 120 min before recalcification.

Inhibitor titers were performed by the Bethesda method. Factor VIII assays for the inhibitor titration were performed by a modification of a one-stage method based on the PTT which permitted the accurate measurement of factor VIII levels as low as 0.2% of normal. One part kaolin-cephalin suspension in barbital buffer and one part of factor VIII-deficient plasma from a patient with severe classic hemophilia were incubated in a glass tube at 37°C for 3 min 45 sec. Then one part of diluted test plasma was added, and after 15 sec the mixture was recalcified and the interval until formation of a clot was timed.

Plasma from 83 patients, including 35 with factor VIII levels of less than 1% (range 0.3%-70%), were tested by this one-stage assay and compared to the two-stage method of Pool and Robinson. The coefficient of correlation (r) was +0.98. Thus we believe this assay to be quite reliable in detecting levels of factor VIII in the range frequently found in hemophiliacs but often not in the range detectable by most one-stage assays.

Subjects

Twenty-five patients with severe classic hemophilia were studied. Fourteen patients had no clinical or laboratory evidence of inhibitors; they served as control subjects. The remaining 11 patients had inhibitor titers between 0.1 and 30 Bethesda units and demonstrable shortening of in vivo survival of infused factor VIII.

RESULTS

Duration of Preincubation

The results of PTTs performed on mixtures of equal parts patient and normal plasma, preincubated at 37°C for various periods, are illustrated in Fig. 1. When mixtures were not preincubated (i.e., 0 time), the PTT of only one inhibitor plasma, with 30 Bethesda units of inhibitor, was longer than 2 SD above the mean for the control subjects. The PTT of a plasma with as much as 20 units of inhibitor was within the limits established for the control group. As the duration of preincubation of the plasma mixtures was lengthened, more inhibitor plasmas could be distinguished from control plasmas. However, even with 120 min of preincubation, inhibitors of less than 0.5 Bethesda units could not be detected consistently.

Ratio of Patient Plasma and Normal Plasma

In order to determine if increasing the ratio of inhibitor plasma to normal plasma in the preincubation mixture would increase the sensitivity of the test, a
ratio of four parts patient to one part normal plasma was arbitrarily chosen for further studies.

The results of the PTTs on a mixture of four parts patient and one part normal plasma preincubated at 37°C for various periods are illustrated in Fig. 2. Without preincubation, inhibitor plasmas with 20 and 30 Bethesda units had PTTs outside the control range, but 11 of 12 inhibitor plasmas with 13.5 and fewer Bethesda units had PTTs in the control range. One plasma, from patient J.S., with 0.4 Bethesda units, had a PTT outside the control range.

With preincubation for 60 min, all inhibitor plasmas with more than 0.5 Bethesda units could be differentiated from control plasmas. Preincubation for as long as 120 min did not suffice for the detection of inhibitor plasmas with less than 0.5 Bethesda units (except for the plasma of J.S.).

**Duration of Incubation of the PTT**

In another variation, patient-normal plasma mixtures were not preincubated but were incubated at 37°C with kaolin-cephalin suspension in glass tubes for
Fig. 3. Comparison of 1:1 and 4:1 mixtures incubated with kaolin-cephalin suspension for 60 and 120 min. Ordinate: PTTs (sec). Area between the horizontal lines encompasses mean ± 2 SD for controls. Circles represent individual PTT determinations for the respective inhibitor units shown within them.

various periods before recalcification. The results are shown in Fig. 3. When equal parts of patient and normal plasma were tested, all inhibitors of more than 0.5 units could be detected after 120 min incubation, whereas the majority of inhibitors of 0.5 units or less could not be detected. In contrast, when four parts patient plasma was mixed with one part normal plasma, all inhibitor plasmas except one (0.4 Bethesda units) could be detected after 60 min incubation and all inhibitor plasmas could be detected after 120 min incubation. It should be noted that a 1-hr incubation of the 4:1 mixture detected more of the mild inhibitors than even 2 hr of incubation of the 1:1 mixture.
DISCUSSION

Many hemophiliacs are treated at hemophilia centers where inhibitor assays are performed by the Bethesda method or similar methods requiring the use of a specific factor VIII assay. However, according to the 1972 Booz-Allen survey of hemophilia care, 58% of hemophiliacs are under the care of a physician who treats less than ten hemophiliacs. Therefore it is important to develop a screening test for inhibitors that is sensitive enough to detect mild inhibitors yet simple enough to be performed in routine clinical laboratories.

This study shows that an easy, sensitive screening test using the PTT can detect even very low titer factor VIII inhibitors. When a patient-normal plasma ratio of 4:1 was used and that mixture was incubated with kaolin-cephalin suspension for 2 hr at 37°C, all of the inhibitors were detectable, including some very weak inhibitors with titers of 0.1, 0.3, and 0.4 Bethesda units. Such weak inhibitors could not be detected with our previous screening technique using equal mixtures of patient and normal plasma and 3 min of incubation of the PTT.

This new screening test is technically within the scope of any clinical laboratory that can perform the PTT. In addition, centers with large numbers of hemophilia patients may prefer to use this PTT screening test in periodic searches for newly emerging inhibitors rather than the more cumbersome Bethesda method, which requires a specific factor VIII assay. As more hemophilia patients are screened for inhibitors with this method, it will be of interest to determine if all inhibitors can be detected by this simple assay.

REFERENCES

Detection of factor VIII inhibitors with the partial thromboplastin time

TS Lossing, CK Kasper and DI Feinstein