CHROMOGENIC SUBSTRATES FOR ACTIVATED PARTIAL THROMBOPLASTIN TIME TESTING: ARE THEY WORTH USING?•

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During the last ten years the use of chromogenic substrates has increasingly challenged clotting methods for hemostasis testing[4]. In some areas, such as determination of single factors and inhibitors, clotting methods have been nearly substituted by chromogenic substrates. However, the new technology has been employed less frequently for global screening tests such as prothrombin time (PT)[2,3,5] and activated partial thromboplastin time (APTT)[1,6], which are still the major tests done in clinical laboratories. During the last two years we have had the opportunity to make a preliminary evaluation of two different reagents for APTT testing that employ chromogenic substrates.

The aim of the present report is to discuss the results obtained and to compare them with those obtained by the traditional clotting method, in order to see whether the chromogenic APTT is suitable for replacing its clotting counterpart.

Reagents investigated

The reagents were studied on two different occasions and were from two manufacturers. For both of them we followed the instructions of the manufacturer closely. Tests with reagent A (Thromboquant APTT, Boehringer, Mannheim, FRG) were run on a completely automated centrifugal analyzer (Cobas Bio, Hoffmann-La Roche, Basel, Switzerland) and the results concerning its evaluation have already been published[6].

Tests with reagent B (Partochrom, Behring Institute, Marburg/Lahn, FRG) were run on a specifically designed instrument (Chromotimer, Behring Institute). The clotting method used for comparison was a conventional APTT which employs micronized silica as activator (Automated APTT, Organon Teknika, Durham, NC). Although the source and the composition of the two

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reagents are different, they share the same methodological principle, which can be summarized as follows: clotting factors of the intrinsic coagulation pathway are activated conventionally by a mixture of cephalin, activator and calcium chloride. The subsequently generated thrombin is then measured with a specific chromogenic substrate. The APTT is the time necessary to obtain a defined increase in the absorbance at 405 nm. The results were expressed as ratios for patient-to-standard plasma run under the same conditions. The methods should be sensitive to deficiencies of all clotting factors of the intrinsic pathway, except fibrinogen.

Issues evaluated

To see whether the new methods may be considered as candidates to replace the conventional clotting method, we looked into the following issues: reproducibility, responsiveness to factors VIII and IX, diagnostic efficacy, responsiveness to heparin, responsiveness to lupus anticoagulant and standardization.

Reproducibility - Between-assay reproducibility was assessed by testing three plasmas with normal, intermediate and prolonged APTT values 20 times in different runs. The coefficients of variation in each case were not higher than 3% for either clotting or chromogenic APTTs.

Responsiveness to factor VIII/IX activities - The responsiveness of chromogenic reagents to decreased amounts of factors VIII and IX were assessed by measuring APTT with plasma prepared by dilution of normal plasma in hemophilic plasma. The prolongations of chromogenic APTT for reagent A and B fell outside the normal range when either factor VIII or IX were lower than 50%. The prolongations were similar, though slightly greater, for the clotting method.

Diagnostic efficacy - The diagnostic efficacy of the chromogenic methods was compared with that of the clotting APTT by testing plasma from patients with mild, moderate and severe deficiency of clotting factors of the intrinsic coagulation pathway, ranging from factor XII to fibrinogen. The diagnosis was regarded as correct when the results obtained by whichever method fell outside the normal range. The clotting APTT correctly diagnosed all the patients under investigation; the chromogenic APTTs correctly diagnosed all except two patients with dysfibrinogenemia and afibrinogenemia. Of course this was to be expected from the principle of the method.

Responsiveness to heparin - The responsiveness of chromogenic APTT to heparinization was assessed for both reagents A and B by testing plasma from patients on heparin therapy and the results compared with those obtained by the clotting method. The correlation between the two sets of results in each case was acceptable, but the slopes of the correlation lines showed a reduced responsiveness of the chromogenic method to heparin. Furthermore, for reagent B the responsiveness was also assessed and compared to that of the clotting APTT by testing a normal plasma to which increasing amounts of heparin, from 0.025 to 0.8 U/ml, had been added. The regression lines obtained with the two sets of results, expressed as the ratio, had different slopes, that of the chromogenic method being 2.5 times lower. The same conclusions were

obtained when the results were expressed as percentage prolongation over the baseline value, and this rules out the possibility that the different responsiveness is a mere reflection of the expression of the results.

Responsiveness to lupus anticoagulant - The responsiveness of chromogenic APTT to the presence of lupus anticoagulant was assessed for reagent A by testing plasmas of 16 patients with known diagnoses of lupus anticoagulant and prolonged APTT. Although the chromogenic APTT was able to detect the presence of the anticoagulant in the majority of the patients, the prolongation was almost invariably less than that obtained with the clotting APTT, suggesting a lower responsiveness to the presence of the anticoagulant.

Standardization - Another issue which would make the chromogenic APTT more appealing than the clotting APTT is its alleged greater amenability to standardization. Although this may be true on theoretical grounds, it cannot be taken for granted in practice because it must eventually overcome at least some of the standardization problems which still afflict clotting APTT, such as cephalins and activators.

CONCLUSIONS

It is claimed that methods that employ chromogenic substrates are more reproducible than their clotting counterparts. This is true for the measurement of single factors and inhibitors, but not for APTT, for which the two methods appeared to be equally reproducible. This is no longer unexpected, since coagulometers of the last generation have achieved a standard of precision comparable to that of clinical chemistry.

An important clinical situation in which the APTT is used is to screen patients for hemophilia. The sensitivity and responsiveness of the chromogenic APTT to factors VIII and IX is satisfactory, being similar to that of the clotting method.

The diagnostic efficacy of the clotting APTT appears to be slightly superior because, unlike the chromogenic APTT, it is sensitive to fibrinogen levels. However, this is not a definite drawback, first because there are other tests for the diagnosis of dysfibrinogenemia and second because clotting APTT in some instance also fails to detect dysfibrinogenemia.

It can be concluded that the performance characteristics of chromogenic APTT are satisfactory as far as the first three issues are concerned and it is therefore able to replace the clotting APTT as a screening test for congenital coagulation disorders. However, in terms of convenience it is, at present, very difficult to justify such a change.

For heparin monitoring and lupus anticoagulant detection, important conditions in which APTT is used, the chromogenic method is perhaps less adequate and an improvement in its responsiveness is desirable.

SUMMARY

In hemostasis testing the development of chromogenic substrates provides an alternative to the traditional methods based on the detection of forming clots. The new technology has often replaced the clotting tests, especially in the area of single clotting factor and inhibitor assay, less frequently for global screening tests. We report studies of the validity and clinical ap-

plication of two reagents for activated partial thromboplastin time (APTT) testing with chromogenic substrates in comparison with the conventional clotting method. Congenital deficiencies of the intrinsic coagulation pathway, other than hypo- and dysfibrinogenemia detected by chromogenic APTT, agreed with those detected by the clotting APTT. The results with the two methods for plasma under heparin treatment suggest a lesser responsiveness of the chromogenic methods to heparinization. The chromogenic methods demonstrated the presence of the lupus anticoagulant in the majority of tested samples of known lupus subjects, but with a lower responsiveness than the clotting method. In conclusion, we found chromogenic APTT suitable for hemostasis testing because it generally gives the same information as the conventional clotting method with the exception of heparin monitoring and lupus anticoagulant detection, where an improved sensitivity would be desirable.

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