Reptilase®/ Batroxobin

Pefakit® Reptilase® Time

Plasma based functional assay

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Biochemistry

Reptilase® (generic name: batroxobin) is 33 kDa proteolytic enzyme derived from the venom of Bothrops atrox (Brazilian lancehead snake). Like thrombin, kallikrein or trypsin, batroxobin belongs to the family of serine proteases. The highly purified enzyme, unlike (diluted) thrombin, is very stable. Reptilase® is free from other enzymatic activities that are found in crude venom or only partly purified preparations that may exhibit different properties.[1]

Reptilase® splits the 16 Arg-17 Gly bond in the A-chain of fibrinogen, releasing the 16-residue peptide fibrinopeptide A (FPA), while thrombin cleaves also at a second site in the B-chain releasing the 14 residue fibrinopeptide B (FPB), a step which is not required for coagulation. The release of FPA by Reptilase® leads to the formation of fibrin I monomer or Des-AA-monomer which spontaneously aggregates into fibrin I clot.

Pathobiochemistry and Application

Fibrinogen degradation products (FDP) induced by the action of plasmin on fibrin in vivo interfere with fibrin polymerization and prolong the clotting time induced by Reptilase®. In contrast to thrombin, Reptilase® does not activate platelets, factor XIII, FVIII or other coagulation factors, and it is not inhibited by antithrombin III.[2] Therefore Reptilase® can also induce clot formation in the presence of heparin or heparin like molecules. Low molecular weight direct thrombin inhibitors (DTI) such as argatroban or dabigatran do not inhibit Reptilase®.
Consequently, the Reptilase® Time assay has found wide application in the haemostasis laboratory. Batroxobin is also used for medical products or pharmaceutical use.

Diagnostic applications

Its unique properties qualify Reptilase® for a variety of applications in haemostasis analysis (table 1). The most frequent one is still the clotting assay Reptilase® Time (or batroxobin time), a useful screening or differential diagnostic test in several clinical situations, specifically for investigating the end-phase of coagulation and fibrin formation. A specific advantage is its insensitivity against heparin or direct thrombin inhibitors.

Batroxobin has also be used in studies on clot retraction and other platelet related functions.[3] Unlike thrombin, batroxobin can also be used for special whole blood assays or tests in platelet rich plasma.[4] Batroxobin is an ideal enzyme for defibrination for assays that cannot be performed in the presence of fibrinogen.

Product

- → Highly purified, stabilized and standardized batroxobin maranhao, lyophilized.
- → Available in 1000 BU/vial. (REF 101-06)
- → Pefakit® Reptilase®: Testkit for the determination of Reptilase® Time. (REF 800191)

Reptilase® Time: a versatile haemostasis assay:

The Reptilase® Time is an important and robust clotting assay for the investigation of the last phase of blood coagulation.[5] Main applications include:

- detection of dysfibrinogenemia (congenital and acquired forms)
- detection of elevated levels of FDPs (e.g. in sepsis, DIC, thromboembolic diseases, liver disease)
- detection of fibrinogen deficiency stages, but also elevated levels

Due to its heparin insensitivity, the Reptilase® Time can detect fibrinogen polymerization disorders, cases of dysfibrinogenemia or fibrinogen deficiency even in the presence of heparin or direct thrombin antagonists such as dabigatran or argatroban. Therefore such disorders of fibrin formations can be detected also in patients during anticoagulant therapy.

Dysfibrinogenemia has a prevalence of almost 1% in patients with venous thrombosis.[6] Dysfibrinogenemia can be induced by mutations in one of the fibrinogen α-, ß- or y- chains (congenital form) or by acquired conditions. Acquired dysfibrinogenemia in patients with liver disease is the most common cause. Up to 50% of patients with severe liver disease secondary to cirrhosis, hepatoma, or hepatitis exhibit bleeding complications.[7]

The prevalence of hereditary dysfibrinogenemia is about 15 per 100,000 of patients undergoing routine coagulation laboratory testing.[8] Clinical consequences of congenital dysfibrinogenemia are quite variable.

Many forms are associated with significant bleeding, e.g. the Denver variant[9], others (less frequently) with thromboembolic diseases, bleeding plus thrombosis, and some are asymptomatic. Some dysfibrinogenemia forms are detected via a prolonged or border line Reptilase® Time, others are abnormal in both Reptilase® and thrombin time tests. In study on 35 patients with hereditary dysfbrinogenemia (65% fulfilled the criteria for a bleeding score of ≥1 in the consensus ISTH bleeding assessment tool), the Reptilase® Time prolongation was more pronounced than the respective thrombin time values.[10] A similar trend was observed in the study on Fibrinogen Šumperk II, a variant that is also associated with bleeding.[11] Elevated fibrinogen, a typical acute phase reaction, may prolong the Reptilase® Time while the thrombin time is less affected.[12] In cases of dysfibrinogenemia, results of different fibrinogen assays may be quite variable.

Fibrinogen degradation products (FDP) lead to a prolongation of the Reptilase® Time.[13] FDPs are found in a variety of diseases in patients with activated coagulation and fibrinolysis, in liver disease and sepsis, specifically in disseminated intravascular coagulation.

	Principle	Clinical situation/diagnostic information provided
Reptilase® Time	Plasma (fibrinogen) + Reptilase® → clotting time	• Thrombosis or bleeding disorders: fibrinogen polymerization disorders (congenital or acquired) • Sepsis/DIC: hyperfibrinolysis, increased levels of FDP • Fibrinogen deficiency or elevation
Reptilase® Time/Thrombin Time combination	Comparison of Reptilase® Time and thrombin time assays (prolonged thrombin time but no effect on Reptilase® Time)	• Detection/exclusion of direct acting thrombin inhibitors such as dabigatran, hirudin or argatroban • Detection/exclusion of heparin • Differential diagnosis of endogenous heparin like anticoagulants, e.g. in liver disease
Fibrinogen clotting assay with Reptilase®	Plasma (fibrinogen) + Reptilase® (excess)→ clotting time	• Quantitative method, similar to Clauss Fibrinogen • No interference from heparin, LMWH, danaparoid, dabigatran or argatroban
Modified thrombelastography ("platelet mapping")	Determination of platelet function (after addition of special agonists and FXIIIa)	• Whole blood method • Detection of platelet dysfunction, e.g. induced by antiplatelet drugs such as acetyl salicylic acid or P2Y12 receptor antagonists
Clot retraction studies	Various methods	• Information on clot retraction and clot stability in various clinical situations • Investigations on platelet gels
Defibrination of plasma	Special methods	• For tests that are only possible in the absence of fibrinogen

Table 1: Important diagnostic applications of batroxobin (summary, see text for details)

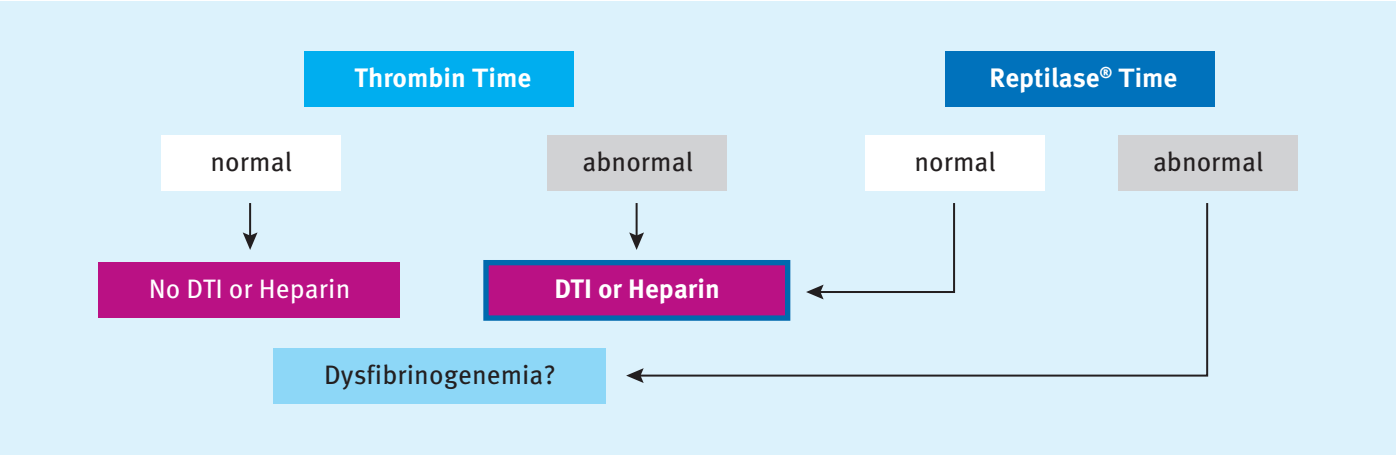

Figure 1: Combined use of Reptilase® Time and Thrombin Time for the exclusion of a potential heparin or DTI effect (a simplified potential diagnostic algorithm)

Reptilase®/Thrombin Time combination: rapid exclusion of several anticoagulants

The combination of Reptilase® Time and Thrombin Time is an inexpensive, rapid and simple qualitative screening test for the detection of several anticoagulants in plasma (or even in whole blood). Anticoagulant substances that are sensitively detected by Thrombin Time but do not affect Reptilase® Time include

- heparin (incl. low molecular weight heparin, though less sensitively)
- direct thrombin inhibitors such as dabigatran or argatroban
- heparinoids with anti-thrombin activity, including endogenous heparin

Detection or exclusion of especially dabigatran, a drug which is now widely used, especially in out-patients, is very important in emergency cases. This drug has only a relatively weak impact on coagulation screening assays such as PT or aPTT, and there may be other causes for abnormality of such tests anyhow. Therefore the results of these two tests can be used for excluding dabigatran and other anticoagulants with anti-FIIa-activity.

An often undetected anticoagulant effect is caused by **endogenous heparin-like substances** which are released from the liver in situations such as sepsis, liver disease,treatment with suramin, or during orthotopic liver transplantation. The combination of Thrombin Time (prolonged) with Reptilase® Time (not prolonged) is useful for detecting such anticoagulant effects and for differential diagnosis with other potential disorders.

Literature

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