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Thrombodynamics, a new global coagulation test: Measurement of heparin efficiency

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ABSTRACT

The actual coagulation status may be reliably measured using only highly sensitive global functional tests; however, they are not numerous and all of them have disadvantages. Thrombodynamics (TD), a novel global coagulation test, is sensitive to hypo- and hypercoagulable states. The main properties of this test were investigated, and its capabilities for hemostasis analysis were verified through pharmacodynamic monitoring of the most widely used anticoagulants, heparins. The anticoagulant effects in the plasma of donors (n = 20) and patients after hip replacement (n = 20) spiked with unfractionated heparin or enoxaparin were measured in vitro to eliminate the influence of pharmacokinetic factors. Sensitivity for heparins was compared for activated partial thromboplastin time, thrombin generation tests and TD. TD was shown to reliably characterize the pharmacodynamics of any heparin in the entire range of its prophylactic and therapeutic concentrations. Inter-individual variability for the anticoagulant action of heparins was also calculated using the TD data. This variability did not differ between the investigated groups and did not exceed 12% and 20% for the stationary clot growth rate in the presence of unfractionated heparin and enoxaparin, respectively. That finding was in accordance with the values determined earlier using the thrombin generation test. The study results showed that TD has advantages over the other global methods of coagulation analysis. These advantages are good standardization, high reproducibility, independence of the parameter values from patient age and gender, and a narrower parameter distribution in a normal population. These results indicate that TD is a promising universal assessment method that improves the quality of hemostasis analysis because it more reliably detects deviations from the parameters' reference values.

Abbreviations: Amax, maximal thrombin concentration; APTT, activated partial thromboplastin time; AT, antithrombin III; CV_{error}, relative random error of measurement; CV_{ii}, inter-individual coefficient of variability; CV_{total}, total coefficient of variation; *ETP*, endogenous thrombin potential; LMWH, low molecular weight heparin; PFP, platelet-free plasma; PPP, platelet-poor plasma ; TD, test of thrombodynamics; TF, tissue factor; TGT, thrombin generation test; *Tlag*, lag-time of clot growth; *t-lag*, time to thrombin concentration of 5 nM; *tmax*, time to maximal thrombin concentration; UFH, unfractionated heparin; *Vi* and *V*, initial and stationary clot growth rate, respectively

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1. Introduction

The aim of this study was to present a new global coagulation test, thrombodynamics (TD), and to show its promise as an accurate assay of coagulation status in the presence of various heparins. Thrombosis is one of the leading causes of mortality worldwide. Anticoagulant therapy is used for the treatment and prevention of thrombotic complications. The proper estimation of hemostasis status is necessary to diagnose the pathology, manage treatment and select adequate doses of anticoagulants. Many tests analyze separate aspects of the coagulation system [1–3]. These tests primarily measure the concentrations of individual coagulation factors and inhibitors or characterize separate subsystems of hemostasis. However, the overall functional activity of the complex and multi-component hemostasis system cannot accurately be described using measurements of the concentrations of the separate system components. Such an assessment requires global (integral) functional tests that measure the total result of all hemostasis reactions under conditions that mimic the situation in vivo [4–6].

Few such methods are available. The most widely known are thromboelastography (TEG) [7–9] and the thrombin generation test (TGT) [10,11]. However, each of these methods has disadvantages. The TEG parameters have wide reference ranges. Additionally, TEG is excessively sensitive to heparins (the most used anticoagulants) and therefore, in many cases, this method is not useful at therapeutic concentrations of these preparations. It is believed that for effective anticoagulant therapy, a dose of heparin should be selected that increases the value of the activated partial thromboplastin time (*APTT*) 1.5–2.5-fold. Concentrations of unfractionated heparin (UFH) and the low-molecular-weight heparin (LMWH) enoxaparin that are > 0.1–0.3 IU of anti-factor Xa activity/ml (ani-Xa IU/ml) completely inhibit clotting in TEG; however, they are too low for therapy and only minimally prolong the *APTT* [8].

The TGT was also demonstrated as a sensitive and promising test for the diagnosis of the coagulation state and the monitoring of therapy in a number of pathologies, in particular to monitor heparins [12–17]. However, currently, this test has many modifications and is not quite standardized. Thus, the development of new global coagulation tests possessing appropriate sensitivity and more standardized and stable parameters is required.

In vivo coagulation develops not only in time but also in space. The process is activated on the surface of a damaged vessel and propagates into its lumen. The novel global coagulation test, thrombodynamics, differs from all other global tests because it simulates the spatially distributed nature of coagulation in the vessels and evaluates the spatial dynamics of clot growth. This test measures the clot size vs. time using light scattering and enables calculation of the spatial clot growth rate to characterize coagulation. To verify the TD test, we used measurements of coagulation efficiency in the presence of various heparins, which are widely used in many pathological states, including cardiovascular diseases, surgery, and post-operative prophylaxis of thromboembolic complications. Heparins act by enhancing the anticoagulant effect of the natural inhibitor of thrombin, antithrombin III (AT) [18].

Treatment with UFH requires constant control of its functional activity because there is high variability in plasma concentrations and functional activity after a fixed dose of UFH in different patients [19]. This unpredictability is due to several reasons, including different plasma levels of AT, individual and nonlinear rates of UFH elimination through two different mechanisms, and binding and neutralization of heparin with different plasma proteins and activated platelets [20–24]. Approximately 10% of people are insensitive to heparin [25]. The nonlinear UFH pharmacodynamics is difficult to predict in advance [26]. UFH is generally measured using the APTT [15] or activated coagulation time (only for high heparin concentrations) [20]. However, these tests are neither highly sensitive nor strictly standardized, and they are practically insensitive to low prophylactic UFH doses. The results of these tests depend on the reagents [27,28] and on the device used for measurement [29]. Consequently, reference values of these parameters should be determined for each particular clinical laboratory, making it difficult to compare the results obtained in different laboratories.

Unlike UFH, LMWHs exhibit more stable pharmacokinetics, and their bioavailability approaches ~ 100% for any dose of subcutaneously introduced preparation [30]. The maximum LMWH concentration in plasma is directly proportional to the LMWH dose [31–33], and many specialists believe that constant coagulation monitoring during therapy is not required because clinical doses of LMWHs may be corrected based solely on the patient body weight [34]. However, the heparin concentration in blood also varied substantially in a healthy population after a fixed LMWH dose, and only partially correlated with the donor body weight [35]. More precise dose correction may be required in many scenarios (e.g., low or high body mass index; critical states; renal failure (creatinine clearance < 30 ml/min); when switching from one anticoagulant to another; age (children and older adults > 75 years) [32,36,37]; and in pregnancy [17]).

The measurement of anti-Xa activity is the most widely used test to manage LMWH therapy [38,39]. However, despite its high sensitivity (the lower limit of determination using a chromogenic substrate is < 0.03 anti-Xa IU/ml [39]), this method is not a global coagulation assay because it measures the concentration of a single factor (Xa) but does not react to AT deficiency or changes in the concentrations of other factors that affect patient hemostasis [15,27]. This method poorly predicts thrombosis or bleeding [27,40].

Thus, a requirement exists for the precise monitoring of any heparin. However, a reliable and universal method for such monitoring is lacking. Our study introduces a novel global coagulation test, thrombodynamics, investigates the main properties of this test and shows that it has advantages compared with the other methods of coagulation analysis in the presence of various heparins.

2. Materials and methods

2.1. Materials

Commercially available Thrombodynamics Analyzer T-2 and corresponding reagent kits and consumables (HemaCore LLC, Moscow, Russia) were used to perform the thrombodynamics test. These kits included cast plastic chambers (HemaCore S.A., Monthey, Switzerland), activators covered with immobilized tissue factor (TF) [41] with a surface density of 90 pmol/m^2, a contact-activation inhibitor (corn trypsin inhibitor) and a calcium acetate reagent.

APTT was measured using the reagent kit Coagulo-test manufactured by Renam (Moscow, Russia). The following reagents were used for the thrombin generation test: the thrombin-specific fluorogenic substrate Z-Gly-Gly-Arg-AMC·HCl (Bachem, Bubendorf, Switzerland); 7-amino-4-methylcoumarin (AMC), $CaCl_2$, and NaCl (Sigma-Aldrich, St. Louis, MO, USA); thromboplastin (Renam, Moscow, Russia); phosphatidylserine (from pig brain) and phosphatidylcholine (from egg yolk) produced by Avanti Polar Lipids, Inc. (Alabaster, Alabama, USA); and 4-(2-hydroxyethyl)-1-piperazine-2-ethanesulphonic acid (HEPES, Fisher Biotech, Fair Lawn, NJ, USA). Medical preparations of UFH (solution of heparin sodium salt, 5000 IU/ml (B. Braun, Melsungen AG, Germany)) and LMWH enoxaparin (sodium salt, 10,000 anti-Xa IU/ml (Sanofi Winthrop Ind., Paris, France)) were used. Activity of TF was measured using Actichrome TF test (American Diagnostica Inc., Stamford, CT, USA).

APTT and TGT were performed in platelet-poor plasma (PPP). TD was measured in platelet-free plasma (PFP).

2.2. Donors and patients

The Ethics Committee of the Center for Theoretical Problems of Physicochemical Pharmacology approved the present study (as part of