NEW ASSAYS FOR MEASURING DIRECT THROMBIN INHIBITORS IN PLASMA

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INTRODUCTION

- Direct Thrombin Inhibitors (DTIs) have increasing and promising curative, preventive or prophylactic applications in severe clinical situations at high risk, and are candidates for substituting to long term oral anticoagulants with vitamin K antagonists.
- Laboratory methods are required for adjustment of drug efficacy and for avoiding overdosage. They must present the most limited impact to other plasma factors (e.g. Antithrombin, Prothrombin, Fibrinogen).
- Ecarin Clotting Time (ECT) and aPTT are useful but too sensitive, insufficiently reliable at high DTI therapeutic levels, and patient coagulation factors may interfere.
- Specialized calibrated clotting and chromogenic assays, fully automatable, with no matrix effect, accurate and sensitive at low and high concentration ranges, were developed for quantitating various DTIs.

METHODS

- Clotting assay (‘Homochot Thrombin Inhibitors’): Sensed thrombin time, using a "substrate" normal plasma pool (R1) mixed with the diluted lost plasma (1:8 to 1:32). Clotting time (CT) is recorded after addition of (n-h) thrombin (R2) containing calcium.
- Chromogenic kinetic assay (‘Biophen DTI’): Tested specimen (1:10 to 1:30) is incubated with thrombin substrate (R1), and (h-n) thrombin (R2) is added. Result is inversely proportional to DTI concentration.
- Aim:
  - To evaluate dose response curves to various DTIs in plasma;
  - To establish accuracy, reproducibility;
  - To compare with a conventional aPTT assay.
- Direct Thrombin Inhibitors tested:
  - Lepirudin (Refludan®);
  - Argatroban®.

RESULTS

- Chromogenic dose response curves with 2 DTIs
- Excellent linearity in the usual therapeutic range for any DTI (Lepirudin, Argatroban®).

Linear regression analysis for measured Lepirudin with both methods (various levels added to normal plasmas)
- Excellent correlation and possible extended dynamic range up to 5µg/ml (useful eg., in ECC).
- NB: For Argatroban®, the clotting assay offers a much higher sensitivity than the chromogenic assay, which is then not appropriate within the normal therapeutic range.

- Excellent performance with Hirudin and analogues, but not suitable for the Argatroban® usual therapeutic range (with low inhibitory potency of thrombin chromogenic activity).

- Measured aPTT on Lepirudin spiked plasma samples (clotting or chromogenic assays), normals or Argatroban® treated patients (clotting only).

GENERAL REFERENCES