Technical method

In vitro assay of thrombolysis with $^{125}$I-fibrinogen

YOSHIRO IGATA, BLEAKLEY CHANDLER Department of Pathology, Medical College of Georgia, Augusta, Georgia, USA

The use of radioiodinated fibrinogen for the in vitro testing of plasmin activity was initially reported by Shulman and Tagnon. Investigators have subsequently used radioiodinated fibrinogen to investigate the fibrinolytic enzyme system and to assay fibrinolytic activity in vitro and in vivo. The principle for the measurement of fibrinolytic activity using radioiodinated fibrinogen is based on the rate of release of radioactivity from the digested $^{125}$I or $^{131}$I-fibrin.

This report describes a radioactive method for assaying thrombolysis in vitro. It was found to be more sensitive, accurate and convenient than the present assay method, which is based on the weighing of fixed thrombi after treatment with thrombolytic agents.

Material and methods

RADIOLABELLING OF THROMBI
Radionuclide-labelled $^{125}$I-fibrinogen (Ibrin, Amsterdam), which contained 160 mCi and 1 mg of human fibrinogen in a vial, was used to label the thrombi. Artificial thrombi were made from citrated blood containing a trace of $^{125}$I-fibrinogen, according to Chandler’s loop method using Connor and Poole’s modified apparatus. In the thrombolytic experiments described below, 70 ml of venous blood were drawn from a healthy donor via a silicone-coated needle into a polypropylene syringe containing 7 ml of 3-8% sodium citrate and 10 mCi of $^{125}$I-fibrinogen. Blood, sodium citrate and $^{125}$I-fibrinogen were immediately mixed. Experiments were conducted within 10 min of collection. One ml of the blood was transferred to a 27 cm length of polyethylene tubing (PE-350, 0.3 cm ID, Clay Adams) into which was added 0.1 ml of 0.25 M CaCl$_2$. A loop was formed by connecting both ends of the tubing with a short collar of tubing, and it was rotated at 12 rpm for 30 min at 37°C to form a $^{125}$I-labelled thrombus. The process was repeated for the number of loops required. The apparatus shown in Fig. 1 can accommodate 80 loops.

Accepted for publication 13 May 1981

RADIOACTIVE METHOD FOR MEASURING THROMBOLYSIS
After sampling 10 µl of residual blood (blood$_1$) from each loop, 0.1 ml of a solution of the thrombolytic agent is added and the loop containing the radio-labelled thrombus is rotated for a given period. If desired, samples of blood can be withdrawn periodically to determine progression of lysis as illustrated in the second experiment described below. Upon completion of the rotation period, the contents of each loop are emptied into a test tube. The residual blood is then decanted and the thrombus is transferred to an aluminium dish and rinsed with 0.9% saline to remove residual blood from the thrombus surface. At the same time, 10 µl of the decanted residual blood (blood$_2$) is sampled. The radioactivities of the blood samples and the thrombus are then measured by means of a gamma scintillation counter. Thrombolysis is calculated according to the formula given below:

\[
\text{Total cpm released into blood} = \text{cpm of original $^{125}$I-thrombus} \times 100
\]

% lysis = \[
\frac{\text{Total cpm released into blood from original $^{125}$I-thrombus}}{\text{cpm of original $^{125}$I-thrombus}} \times 100
\]

Radioactivity (cpm) of original $^{125}$I-thrombus = cpm of partially lysed thrombus plus total cpm released into blood from original $^{125}$I-thrombus [A]

\[A = \frac{\text{cpm of blood$_2$ \times total volume (µl) in loop}}{10 \text{ µl}} - \frac{\text{cpm of blood$_1$ \times total volume (µl) in loop}}{10 \text{ µl}}\]

COMPARISON OF WEIGHING AND RADIOACTIVE METHODS
In this experiment, $^{125}$I-thrombi were divided into four groups: saline, and urokinase (UK) in final concentrations of 37.5 IU, 75.0 IU and 150.0 IU/ml of blood, respectively (high molecular weight lyophilised urinary UK was kindly supplied by Green Cross Corp). Nineteen thrombi were in the saline control group and 9 in each of the UK groups. After sampling 10 µl of blood (blood$_1$) from each loop, 0.1 ml of saline or urokinase solution in normal saline was added. The loops were rotated at 12 rpm, for 4 h at 37°C. Samples (10 µl) were drawn from the residual blood (blood$_2$) from each loop and the separated thrombi were rinsed in saline. Fixation of the thrombi in Bouin’s solution followed. After 16 h, the thrombi were removed from the fixative,
blotted and weighed. The radioactivities of the fixed thrombi and blood samples (blood1 and blood2) were measured and the percent lysis was calculated according to the formula given above. Calculation of the percent lysis of a thrombus by the weighing method was performed according to the equation given below:

\[
\% \text{ lysis} = \left(1 - \frac{\text{Weight of treated thrombus}}{\text{Mean weight of control thrombi}}\right) \times 100
\]

**TIME-COURSE OF THROMBOLYSIS**
In this experiment, two \(^{125}\text{I}\)-thrombi were rinsed with saline and their radioactivities were counted. They were then separately transferred to new loops in each of which was added one ml of fresh citrated blood followed by 0·1 ml of normal saline in one loop and 0·1 ml of urokinase solution in the other loop. The loops were rotated at 12 rpm for 4 h at 37°C. During rotation a 10 µl blood sample was taken at 1, 2, 3, and 4 h after the addition of UK or saline. At the end of 4 h, the radioactivities of the blood samples and each thrombus were measured.

**Results**

**COMPARISON OF WEIGHING AND RADIOACTIVE METHODS**
Table 1 shows the mean radioactivity (cpm) and weight (mg) of the thrombi in the saline group. The coefficient of variability for cpm was 5·2%, or half of the value for mg weight. The correlation between cpm and mg was \(-0·13\), suggesting no correlation between cpm and mg weight of each thrombus.

Table 2 shows the mean radioactivity (cpm), weight (mg) and \% lysis by the radioactive (RA) and weighing (WT) methods for partially lysed thrombi treated with urokinase for 4 h at final concentrations of 37·5 IU/ml, 75·0 IU/ml and 150·0 IU/ml, respectively. The mean weight of the saline thrombi, as shown in Table 1, was used as a control value for calculating percent lysis of the UK groups according to the weighing method.

**Table 1** Comparison of mean radioactivity (cpm) and weight (mg) of thrombi in a saline control group

<table>
<thead>
<tr>
<th>Mean radioactivity (cpm)</th>
<th>Mean weight (mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No thrombi</td>
<td>19</td>
</tr>
<tr>
<td>Mean</td>
<td>81060</td>
</tr>
<tr>
<td>Range</td>
<td>75613-92884</td>
</tr>
<tr>
<td>SD</td>
<td>4172</td>
</tr>
<tr>
<td>c</td>
<td>5·2%</td>
</tr>
<tr>
<td>r</td>
<td>(-0·13)</td>
</tr>
</tbody>
</table>

SD = standard deviation; c = coefficient of variability; r = correlation coefficient.
Table 2  Comparison of radioactive (RA) and weighing (WT) methods for estimating thrombolysis in vitro induced by urokinase (UK)

<table>
<thead>
<tr>
<th>Group</th>
<th>Original thrombus</th>
<th>Residual blood</th>
<th>Thrombus after 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total radioactivity (cpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>37.5 IU/ml UK</td>
<td>75.0 IU/ml UK</td>
<td>150.0 IU/ml UK</td>
</tr>
<tr>
<td>Thrombi</td>
<td>RA mg</td>
<td>RA mg</td>
<td>RA mg</td>
</tr>
<tr>
<td>cpm (n = 9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>72203</td>
<td>30403</td>
<td>12972</td>
</tr>
<tr>
<td>Range</td>
<td>36-6935 - 10072-8393</td>
<td>11-7-235 - 776-932</td>
<td>1402</td>
</tr>
<tr>
<td>SD</td>
<td>0-44t</td>
<td>0-03t</td>
<td>0-84t</td>
</tr>
<tr>
<td>r</td>
<td>0-03t</td>
<td>0-68t</td>
<td>0-84t</td>
</tr>
</tbody>
</table>

SD = standard deviation; c = coefficient of variability; r = correlation coefficient.

Table 3  Time-course of thrombolysis by urokinase using the radioactive method

<table>
<thead>
<tr>
<th>Group</th>
<th>Original thrombus</th>
<th>Residual blood</th>
<th>Thrombus after 4 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Total radioactivity (cpm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 h</td>
<td>1 h</td>
<td>2 h</td>
</tr>
<tr>
<td>Urokinase</td>
<td>193 479</td>
<td>720</td>
<td>138 160</td>
</tr>
<tr>
<td>150 IU/ml</td>
<td>100%</td>
<td>0-37%</td>
<td>71-4%</td>
</tr>
<tr>
<td>Saline</td>
<td>220 400</td>
<td>1700</td>
<td>1500</td>
</tr>
<tr>
<td>control</td>
<td>100%</td>
<td>0-77%</td>
<td>0-68%</td>
</tr>
</tbody>
</table>

Percentages indicate percent of total radioactivity of original thrombus.

Discussion

The radioactive procedure here described provides a sensitive assay for thrombolysis in vitro. The previously used weighing method is not sensitive...
Technical method

enough to detect slight lysis resulting from low concentrations of lytic agents since individual variation in the control group may at times give lower weights than the experimental group. On the other hand, there is good correlation between the two procedures under conditions of greater lysis. The weighing method reflects, not only lysed fibrin, but also the release of materials from the thrombus other than fibrin. Since fibrinolysis is the process of chief interest in the study of thrombolysis, the radioactive assay, by virtue of its inherent mechanism, would seem to be the most appropriate.

With this assay method, the test thrombus serves as its own control so that the time-course and kinetics of thrombolysis can be readily measured over a predetermined schedule. It is concluded that the radioactive method is a reliable, sensitive and rapid procedure for estimating thrombolysis in vitro.

We thank Susan Johnson and Sylvia Greenwald for their technical assistance and Marie Hiller for preparing the typescript.

References


Requests for reprints to: Dr AB Chandler, Department of Pathology, Medical College of Georgia, Augusta, Georgia 30912, USA.
In vitro assay of thrombolysis with 125I-fibrinogen.

Y Iga and A B Chandler

*J Clin Pathol* 1981 34: 1400-1403
doi: 10.1136/jcp.34.12.1400

Updated information and services can be found at:
http://jcp.bmj.com/content/34/12/1400.citation

**Email alerting service**

Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

**Notes**

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/