Regional thromboplastin standardisation using a human brain extract

A. R. BIRD, B. KOSSEW, T. P. MULLIGAN, P. JACOBS

Summary

The preparation, regional distribution and standardisation of an acetone-dried extract of human brain thromboplastin was prospectively evaluated. The programme was shown to be easy to implement within the constraints of the World Health Organisation recommendations and offers a practical alternative to a national standard in countries where the cost of distribution and lack of expertise in preparing such material are limiting factors.

The World Health Organisation scheme for standardisation of prothrombin time determination to control oral anticoagulant dosage was proposed in 1982 after a critical appraisal of the Biggs-Denson calibration model, which was shown to be invalid when markedly dissimilar thromboplastins were compared. An alternative calibration method was formulated and tested, with better results, in a study organised by the Community Bureau of Reference (CBR) of the European Communities. The latter has now been widely adopted and most laboratories participating in the International Committee for Standardisation in Haematology Task Force Quality Control Programme currently report the prothrombin time as the international normalised ratio (INR). In the USA support for similar standardisation is gaining acceptance, despite earlier reservations.

To explore the feasibility of a regional standardisation scheme, a locally prepared acetone-dried extract of human brain was calibrated as a reference preparation and used to standardise five different thromboplastin preparations from seven laboratories in hospitals serving an area of 100 km² with a population of approximately 2 million.

Material and methods

The regional standard was prepared every 4 months from human brain, acetone-dried and stored at -20°C in ± 3 g aliquots in plastic containers. Working preparations were reconstituted in phenol and stored at 0-4°C. Prothrombin times were measured with a Clotek II (Hyland Diagnostics, USA). The regional standard was calibrated against an international primary reference preparation for human thromboplastin (BCT/253) from the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service or, alternatively, against a secondary reference preparation from the CBR in Brussels, according to WHO recommendations. Prothrombin times were determined for 60 patients in good general health who had been stabilised on coumarin anticoagulants for at least 6 weeks. On each day 6 patients plus 1 normal male and 1 normal female subject were tested, using each thromboplastin. The international sensitivity index (ISI) of the working reference preparation (WRP), in this case our regional standard, was obtained by plotting the prothrombin time on logarithmic axes for the reference and test thromboplastins, fitting a straight line, and estimating the slope using the technique of orthogonal regression. The ISI of the regional standard (WRP) is obtained as follows:

\[ \text{ISI}_{\text{WRP}} = \frac{\text{ISI}_{\text{RP}} \times \text{IRP} \times \text{WRP}}{\text{c}} \]

where \( c \) = the slope of the regression curve and IRP = international reference preparation, primary or secondary.

The recommended coefficient of variation (CV) of the estimation should be 3% or less, although a CV of less than 5% may be acceptable in certain circumstances. To convert the prothrombin time ratio (PR) of any given thromboplastin to the INR the following formula is used: \( \text{INR} = \frac{\text{PR}}{\text{IRP}} \).

This conversion can be achieved on any scientific calculator with a \( y^x \) function, and tables are easily prepared in the laboratory. Alternatively, the formula \( \text{INR} = \frac{\text{antilogarithm} (\text{ISI} \times \log \text{PR})}{10} \) may be used.

Thromboplastins from regional laboratories were calibrated according to the procedure recommended by the WHO, using batches of frozen pooled coumarin plasma and pooled normal plasmas. A minimum of 15 separate prothrombin time measurements were performed with each thromboplastin in parallel with the regional standard, which now functioned as the reference preparation. In some cases comparisons were made with the ISIs obtained by testing 20-25 fresh coumarinised plasmas concurrently with the local reference preparation.

As a further form of quality control, comparisons were made between the INRs obtained for both normal persons and patients on regular coumarin therapy, using a rabbit calcium thromboplastin (Instrumentation Laboratories, USA; ISI = 2.3) and our regional standard, on both the Clotek II (Hyland Diagnostics, USA) and the ACL Automated Coagulation Laboratory (Instrumentation Laboratories, USA).

Results

Three batches of the local reference standard have been studied since 1985 and the ISI values are given in Table I, together with other thromboplastins. The local reference reagent showed little variation over the three batches, the ISI ranging from 1.31 to 1.49, which was similar to the other two acetone extracts. The remaining thromboplastins had ISIs at expected levels of sensitivity in that the rabbit brain was relatively insensitive at an ISI of 2.0, while the human extracts were more sensitive with ISIs at ± 1.0. The acetone extract obtained from one brain provided sufficient thromboplastin for 200 prothrombin time estimations daily over a 4-5-month period, with 75% of these carried out in the university hospital and the remainder between two private laboratories.

The INR values obtained using the regional standard on two different automated coagulation machines (Clotek II and the Automated Coagulation Laboratory) were compared in 218 samples, of which 29 were derived from normal subjects.
and the remainder from individuals on regular coumarin therapy. The correlation was excellent \( r = 0.98; P < 0.01 \). The regional standard and the commercial calcium rabbit brain thromboplastin were compared in 88 patients on regular coumarin therapy, using the Clotek II for estimating prothrombin times with the regional standard and the ACL machine for the rabbit thromboplastin. This also showed good correlation \( r = 0.93; P < 0.01 \). When the ACL machine alone was used with both thromboplastins in the same group of patients, close correlation was again demonstrable \( r = 0.97; P < 0.01 \). In 9 patients (10%) the dosage of coumarin would have been different when the two reagents were compared using the alternative machines, but only minor alteration in anticoagulant dosage would have been required in each case. The stability of our regional standard should ideally have been tested by performing regular ISI estimations against an IRP, but this was precluded by cost and limited supply of the reagents. Prothrombin times were determined on commercial reference plasmas (normal, midrange, and abnormal; Hyland Diagnostics, USA) at least twice weekly, and the monthly means with standard deviations, plotted over a 5-month period, are shown in Fig. 1. There is a minor variation in the normal and midrange reference plasmas, with a more pronounced fluctuation in the abnormal plasma.

**Discussion**

The WHO recommends that oral anticoagulant therapy is ideally controlled by means of a national system employing a standardised thromboplastin reagent. This situation has, however, been implemented in relatively few countries, since the establishment of a standard that is stable and available in sufficient quantities is technically demanding and time-consuming. As a less preferable alternative, the WHO suggests that regional standards be established. However, given the difficulties in preparing suitable national standards our experience would suggest that regional standardisation is a realistic alternative. Although it is emphasised that a high degree of technical and statistical expertise is required to calibrate a reagent, the availability of reliable automated instruments has increased the precision of prothrombin time determination. Moreover, the widespread use of microcomputers makes the statistical calculations relatively straightforward. As can be seen from Table I, the CV for the estimation of the ISI was satisfactory in most cases, always being 3% or less when calibrating the regional standard. In the batch calibrations, using pooled frozen plasma samples, it was less than 5% in the majority of cases, although there were two exceptions. For individual batch calibration, a CV equal to or less than 5% would therefore seem reasonable.

Larger quantities of thromboplastin can be obtained with the acetone-dried extract than with the saline extract, and it is conveniently stored in its dried form and takes up little space. The stability of the preparation is satisfactory (Fig. 1), in contrast to some previous reports. Other thromboplastins could presumably function as stable regional reference preparations in a similar fashion; a commercially manufactured thromboplastin with an established ISI could easily be used as a standard for regional hospitals intending to calibrate their home-made thromboplastins.

At present, human brain preparations are being phased out in the UK. This follows concern that human immunodeficiency virus could be transmitted to persons preparing the thromboplastin (L. Poller — personal communication). However, there have been no cases reported of laboratory personnel contracting such infections during these procedures. Although a sensitive rabbit brain thromboplastin has been prepared in the UK, a comparable animal product is not available locally at present. Until it is we shall continue to use the human product and take the precaution of checking for HIV antibodies in the donor and restricting all preparative procedures to a biohazard cabinet.

In summary, this study supports the recommendations of the current WHO calibration scheme for anticoagulant control and may at last resolve the hitherto rather unsatisfactory state of affairs regarding thromboplastin standardisation. In countries where the establishment of national standards is difficult, the use of regional standardisation schemes similar to the programme described can be implemented relatively easily.

**TABLE I. ISI RESULTS OF THE REGIONAL THROMBOPLASTINS**

<table>
<thead>
<tr>
<th>Batch No.</th>
<th>ISI</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regional standard</td>
<td>2/85</td>
<td>1,381</td>
</tr>
<tr>
<td></td>
<td>1/86</td>
<td>1,378</td>
</tr>
<tr>
<td></td>
<td>2/86</td>
<td>1,310</td>
</tr>
<tr>
<td></td>
<td>1/87</td>
<td>1,490</td>
</tr>
<tr>
<td>Laboratory A (human, saline)</td>
<td>3/85</td>
<td>1,052</td>
</tr>
<tr>
<td></td>
<td>1/86</td>
<td>1,139</td>
</tr>
<tr>
<td></td>
<td>2/86</td>
<td>1,263</td>
</tr>
<tr>
<td>Laboratory B (human, acetone)</td>
<td>1/85</td>
<td>1,547</td>
</tr>
<tr>
<td></td>
<td>1/86</td>
<td>1,416</td>
</tr>
<tr>
<td>Laboratory C (human, acetone)</td>
<td>1/85</td>
<td>1,452</td>
</tr>
<tr>
<td></td>
<td>1/86</td>
<td>1,122</td>
</tr>
<tr>
<td>Laboratory D (Simplastin II)</td>
<td></td>
<td>2,043</td>
</tr>
<tr>
<td>Proplastin (human, acetone)</td>
<td></td>
<td>0,903</td>
</tr>
<tr>
<td>SAIMR (human, saline)</td>
<td></td>
<td>1,233</td>
</tr>
</tbody>
</table>

*The reason for the high CV in these two calibrations was not clear. The substitution of fresh coumarinised plasma for frozen coumarinised pools for secondary calibration made little difference to the ISI or CV in the three instances where they were compared.

SAIMR = South African Institute for Medical Research.

**Fig. 1. Stability of the regional standard.** Prothrombin times were determined at daily intervals or sometimes on alternate days, on normal, midrange and abnormal plasma (Hyland Diagnostics, USA). The monthly means and standard deviations are shown.
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REFERENCES

Termination of pregnancy with mifepristone after intra-uterine death

Clinical and hormonal effects

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Summary

Mifepristone was used in a dosage of 400 mg/d in a double-blind study to induce labour in patients with intra-uterine fetal death in late pregnancy. Eight of 12 patients who received the drug delivered within 72 hours while only 2 of 12 patients treated with placebo delivered during a similar period. No adverse effects, viz. excessive vaginal bleeding and abnormal biochemical or haematological parameters, were associated with the use of this drug.


Mifepristone, a derivative of the progesterone, norethindrone, has been shown to have potent antiprogesterone activity both in vivo and in vitro. Administration of this drug in the luteal phase of the menstrual cycle induces menstruation by a local action on endometrial progesterone receptors, while its use as an abortifacient in the 1st trimester of pregnancy has been well established in numerous trials reflecting the dependence of early pregnancy on progesterone. The action of this drug in late pregnancy, however, has not been evaluated in a controlled double-blind study because of potentially harmful effects on the fetus resulting from the antiglucocorticoid action.

A study was undertaken to determine whether mifepristone was effective in inducing labour in patients with intra-uterine fetal death in the second half of pregnancy.

Patients and methods

Consent for the study was obtained from the Ethics Committee of the Faculty of Medicine, University of Natal. Twenty-four patients participated in a double-blind controlled study. All patients were over the age of 18 years, had amenorrhoea > 26 weeks duration and had no evidence of ongoing labour. Patients had no clinical or biochemical evidence of hepatic, renal or adrenal disease and had not received any corticosteroids during or before pregnancy. All 24 patients had fetal intra-uterine deaths confirmed ultrasonographically and were inpatients at King Edward VIII Hospital, Durban.

Before drug therapy, cervical status was assessed using Bishop's score. Each patient then received a coded bottle containing tablets (mifepristone 200 mg/tablet or placebo) to be taken at a dose of 1 tablet twice daily for 3 days. The drug was stopped if the patient went into labour in under 72 hours. Patients were examined each morning and evening during which time cervical status was assessed. Success of treatment was defined as vaginal delivery within 72 hours of ingestion of the first tablet. Patients who did not deliver during this time were regarded as failures. The codes for each of the patients were only known on completion of the study.

Investigations performed included a full blood count, measurements of serum urea, electrolyte, creatinine, aspartate transaminase, γ-glutamyl transferase and glucose levels and partial thromboplastin times before and after induction of labour. Venous samples for total progesterone, oestradiol,