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Standardization of Bovine Lung Thromboplastin according to the WHO Report: Calculation of the ISI Value and the Prothrombin Time as INR

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Thromboplastin is an important reagent for prothrombin time (PT) test. Various types of thromboplastin are prepared commercially and it is essential that each reagent is correctly standardized, in order to be able to interpret the results of the prothrombin time test. This will ensure that the results of tests with different products and batches are reproducible and can be compared. For this reason we prepared thromboplastin extract from bovine lung and calibrated according to the thirty-third report of the WHO Expert Committee on Biological Standardization. With this procedure, standardization parameter called International Sensitivity Index (ISI) was calculated and it is used for converting the PT values from seconds to International Normalized Ratio (INR). By this calculation it is possible to compare the PT results, which were performed by different thromboplastins. After standardization we used this thromboplastin extract for PT test and compared this extract with the commercial kit for PT test. In conclusion the results of our evaluation show that our thromboplastin extract can be used safely as a reagent for PT tests instead of commercial PT kit.

Keywords: Bovine lung, thromboplastin, ISI, INR, prothrombin time

1. Introduction

The protrombin time (PT) is the clotting time of plasma sample in the presence of a preparation of thromboplastin and the appropriate amount of calcium ions [1]. It is used to measure the acquired or hereditary coagulation system deficiencies and especially to monitor the anticoagulant therapy [2-4].

Various types of thromboplastin which are used for PT test, are prepared from bovine, porcine, rabbit and human brain, lung, placenta and by recombinant techniques. Thromboplastin (tissue factor, FIII) is an integral membrane protein functioning as a cofactor enhancing the proteolytic activity of factor FVIIa towards factor X and factor IX in the blood. Its protein, phospholipid and carbohydrate content differ from species to species [5,6].

PT test is controlled by the use of calibrated thromboplastins and plasmas. In order to be able to interpret the results of the prothrombin time test, it is essential that each reagent is correctly calibrated. This will ensure that the results of tests with different thromboplastin preperations are reproducible and can be compared. A procedure for the calibration of thromboplastins using a logarithmic plot of protrombin times has been developed [7] and was described in the thirty-third report of the WHO Expert Committee on Biological Standardization [3]. With this procedure, the definition of calibration parameter called International Sensitivity Index (ISI) became feasible. It is possible to express prothrombin time results on common scale, i.e. the International Normalized Ratio (INR), if the ISI of the thromboplastin used is known [3,4,7,8].

In this study we prepared thromboplastin extract from bovine lung and calibrated according to the World Health Organization (WHO) reports [3,4] and used for prothrombin time test and compared this thromboplastin extract with the commercial PT kit.

2. Material and Method

2.1. Preparation and Standardization of Bovine Lung Thromboplastin Extract

Fresh bovine lung was obtained from Coskun Et and Et Mamulleri and was brought to our laboratory in the ice box. Lung is washed with saline solution (0,9 g % NaCl). Membranes and blood vessels were cleared from the lung and were cut in to very small pieces . These small pieces weighed

Table 1
Comparison of INR values

	Bovine lung thromboplastin extract		Commercial kit (Thromborel S)		
PT (INR)	Ort ($n = 100$)	SD	Ort ($n = 100$)	SD	P_{t-test}
	1.39	0.85	1.38	0.81	0.136

and mixed with equal amount of saline solution (0.9 g % NaCl). This suspension is mixed every 10 minutes for 3 hours. It was kept in refrigerator ($+4\,^0C$) overnight and filtered from cheese cloth folded three times. The filtrate was aliquoted (3 mL) and kept in the freezer (-20 ^0C) until the day it was used [9,10].

The calibration procedure entails the determination of a series of prothrombin times, using normal and coumadin plasmas, with both the reference and test thromboplastin [11,12]. Blood samples were obtained from Metropolitan Florance Nightingale everyday for 10 days. Subjects were selected from 3 healthy subjects and 6 patients who have been on oral anticoagulants (coumadin) for at least 6 weeks. Prothrombin times of these subjects were tested immediately in our laboratory both with our bovine lung thromboplastin extract and with International Reference Preperation (IRP) of bovine thromboplastin (OBT \79, ISI $_{IRP}$: 1.0) which was obtained from WHO. PT is assayed according to the method of Quick [1].

The ISI value of our thromboplastin is calculated with the orthogonal regression analyze as follows :

$$ISI_{WRP} = ISI_{IRP} \times C_{IRP,WRP} \tag{1}$$

$$C_{IRP,WRP} = m + \sqrt{m^2 + 1} \tag{2}$$

$$m = \frac{A - B}{C}, \tag{3}$$

where

$$A = \sum (LPT_{IRP} - LPT^*_{IRP})^2 \tag{4}$$

$$B = \sum (LPT_{WRP} - LPT^*_{WRP})^2 \tag{5}$$

$$C = 2\sum (LPT_{IRP} - LPT^*_{IRP}) \times (LPT_{WRP} - LPT^*_{WRP}) \tag{6}$$

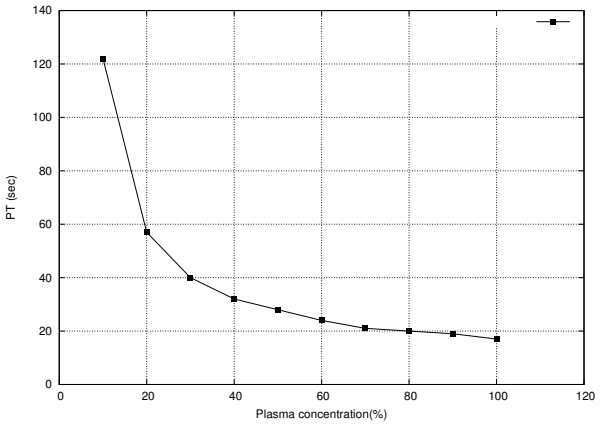

Figure 1. Reference curve.

IRP = International reference preperation (obtained from WHO)

WRP = Working reference preperation (that is our thrombolastin extract)

LPT_{IRP} = Logarithm of individual prothrombin time using the Internatioanal Reference Preparation

LPT^*_{IRP} = Mean of logarithms of individual prothrombin times using the International Reference Preparation

LPT_{WRP} = Logarithm of individual prothrombin time using the working reference preparation

LPT^*_{WRP} = Mean of logarithms of individual prothrombin times using the working reference preparation

$\sum$ Denotes summation of terms for all plasmas (normals and patients).

We used this ISI value for the calculation of International Normalized Ratio (INR)

$$INR = \left(\frac{K}{L}\right)^{ISI}, \tag{7}$$

where K: PT value of the individual (sec) and
L: Mean value of healthy individuals PT value(sec)

2.2. Establishment of the Reference Curve

Plasma samples of ten healty subjects were diluted as %10, 20, ...%100 with saline solution (0.9 %g NaCl). The PT of the diluted plasmas determined by bovine lung thromboplastin extract and plotted versus plasma dilutions, thus hyperbolic reference curve is obtained. According to

Table 2
Correlation Analyze. * p < 0,0001

Bovine lung thromboplastin extract	Coomercial kit (Thromborel S)	Correlation coefficient
PT(seconds)	PT(seconds)	$r = 0,992^*$
PT(INR)	PT(INR)	$r = 0,994^*$

this curve, it is decided whether thromboplastin extract can be used for PT test or not. Furthermore, by using this curve, PT test results can be reported as the activity of prothrombin in the blood. Higher plasma dilutions result in longer prothrombin times.

2.3. Comparison of PT

100 subjects were randomly selected from healty subjects and oral anticoagulant users who attended to Şişli Etfal Hospital and Metropolitan Florence Nightingale Hospital.

PT times were performed with the bovine thromboplastin extract which was calibrated and with the commercial PT kit (Thromborel S, Dade Behring, Kat.No: OUHP35). PT values (sec) were converted into INR [2,13,14,15] and compared.

Statistical evalutions were determined by using Unistad 5.0 package program. Correlation analysis and student "t" test were performed.

3. Results

ISI value of bovine lung thromboplastin which was calibrated with WHO reference standart OBT\79 by using orthogonal regression analysis was 1.20. Reference curve of bovine lung thromboplastin is given on Fig. 1.

As it is seen from the reference curve (Fig. 1) PT got longer as plasma dilution increased. This hyperbolic curve is suitable to distinguish the difference between normal and abnormal PTs.

There was no significant different between the PT values as INR's of the 100 individuals that were performed by both commercial PT kit and bovine lung thromboplastin extract ($p_{t-test} = 0,136$) (Table 1).

There was strong positive correlation between the PT values (seconds or INR), which were determined by using commercial PT kit and bovine lung thromboplastin extract (r=0,992, r=0,994) (Table 2).

4. Discussion

Thromboplastin is a material that is used for the evaluation of extrinsic coagulation system. In commercial kits it is prepared from various sources including rabbit brain, human placenta or by recombinant techniques [2,7].

It is well established that different thromboplastins respond to PT test with different clotting times, thereby making it difficult to compare the results obtained in different laboratories [16]. Weiss et al. revealed that the blood and inhibitor content of the lung and brain thromboplatin extracts change the PT test results [17]. A considerable improvement in the comparability of results however can be achieved by reporting the PT results as INR [4]. To this end, working thromboplastins must be calibrated against International Reference Preperations.

In this study we calculated the ISI value of our thromboplastin extract according to the WHO calibration protocol. Recently it is easy to identify the more sensitive reagents on the basis of the ISI, which spans from 1.0 for the most sensitive to 2.0 or even higher for the least sensitive. The ISI value of our thromboplastin extract was found as 1.20 and its sensitivity was suitable for PT tests.

When we compared the PT test results which were performed with both commercial kit (Thromborel S, Dade Behring, Kat.No:OUHP35) and bovine lung thromboplastin extract, the difference between INR values is not significant, that shows the suitability of our thrombopastin extract for PT test. Also there was a significant correlation between PT test when they were presented as seconds or as INR.

In conclusion the results of our evalution show that our thromboplastin extract can be used safely as a reagent for PT tests instead of commercial PT kit. Preparation cost of our thromboplastin extract is cheaper than the commercial thromboplastins and this can prevent the lost of foreign exchange.

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