Usefulness of Plasma Calibrant for Monitoring of Oral Anticoagulant Therapy

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Abstract

The values of prothrombin time (PT) and thrombotest (TT) were examined comparatively for monitoring of oral anticoagulant therapy by the calibration curve using Internal Normalized Ratio (INR) calibration plasma. There were good correlations in both the whole treatment area and the high treatment area (TT 15% or less). On the other hand, differences among 4 lots of the prothrombin reagent were obvious as PT was in the abnormal area shown in the expression of seconds. Also, since the use of the INR calibration plasma is convenient, mean normal protoronbin time (MNPT) can be calculated simply. Therefore, giving INR report by using calibration plasma for the monitoring of oral anticoagulant therapy is the most suitable and practical method for the standardization of INR, TT as well as PT.

Key words: anticoagulant therapy, monitoring, INR calibration plasma, prothrombin time, thrombotest

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Ⅰ. Introduction

In the monitoring of oral anticoagulant therapy, prothrombin time (PT) and thrombotest (TT) are used. In modern society there has been a tendency toward an increase in patients with an indication of oral anticoagulant therapy and in addition, data compatibility among medical institutions is required, since migration of population is intense. However, it was not possible to obtain interchangeable data due to the interlaboratory difference, because the thromboplastin reagent on the market is an organ extraction of phospholipid pharmaceutical.

Therefore, WHO established an International sensitivity index (ISI) of the PT reagent in 1983, with the recommendation that it was described by the International Normalized Ratio (INR) [1].

Though it has been more than ten years since the system was introduced worldwide as recommended, the PT/INR report has not spread because many laboratories have adopted the TT system in Japan. Further, from results of external quality control survey, it is known that the dispersion becomes small in comparison with other notation on the INR display, the values do not always agree. In addition, it might be inaccurate for ISI display attached to each reagent, many confusions occured. With the recognition of the system, it is also indicated that INR is affected by the combination of the reagent and measuring device and setting of the PT average second number (mean normal protoronbin time : The following MNPT) for healthy subjects. The present situation is that the state has not been reached to obtain uniform data from the results of past in external quality control surveys. A recent report shows that lyophilized plasma with assigaed value of INR, which is available from several companies on the market, is useful for the solution of the above described problem. In this paper, we describe the comparison of PT-INR results and TT-INR results using INR calibration plasma in patients with therapeutic threshold value of oral anticoagulant therapy.

Ⅱ. Subjects

The subjects were 144 oral anticoagulant therapy patients (83 men, 61 women), who received medical examination from May 2001 to July 2001.

The sample was the patient plasma (with 3.13% sodium citrate added), which was used after centrifugationl in order to produce platelet poor plasma (PPP).

Ⅲ. Reagent and equipment

1. Reagent

1) PT reagent

Thromborel S (DADE Behring) : The human tissue extract thromboplastin

2) TT reagent

The compound factor TTO (Kokusai) : An extract of bovine brain derivation thromboplastin and Calcium lactate

3) INR calibration plasma kit (DADE Behring) (Table 1)

* INR plasma (B, C, D) were prepared by freeze-drying of the plasma of patients who were receiving long-term oral anticoagulant therapy.

The compound factor (Ⅱ, Ⅶ, Ⅸ, Ⅹ, PC, PS) is low-valued, and PIVKA is contained.

2. Eequipment

Sysmex CA-500

Table 1 INR calibration plasma kit (DADE Behring)

	plasma	INRvalue	PTactive%	TTactive%
INR plasma A	Normal	1.0	100	100
INR plasma B	Low abnormal	2.0	40	20
INR plasma C	Abnormal	3.0	20	10
INR plasma D	High abnormal	4.0	10	6

Ⅳ. Methods

1. Calibration curve preparation by the INR calibration plasma kit. (PT, TT).
   1) The dissolution reagent was prepared according to the given method, and each INR value and activity value were entered manually into CA-500.
   2) Each plasma sample was measured twice, and the coagulation time (second) was decided.
   3) Local SI value and MNPT were required, using the calculation tool.
2. Measurement of the sample (PT, TT)
   1) The sample was measured twice, and the mean value was made to be the measurement result, and coagulation time (second), active %, INR value were calculated. (Automatic measurement was done after calibration curve input.)
   2) Regression line and correlation coefficient were obtained from PT-INR, TT-INR of each sample.
3. Measurement of INR calibration plasma by 4 Lot PT reagents.
   1) PT reagents of 4 Lots were adjusted, INR calibration plasma (A, B, C, D) was measured for each Lot reagent twice, and coagulation time (second), active % and INR value were obtained.
   2) The CV on the data were compared, and mean value and fluctuation range (minimum value, maximum value) were graphed for each coagulation time (second).

Ⅴ. Results

1. The correlation of PT-INR and TT-INR in the treatment area gave correlation coefficient of r=0.986, and regression formula of y=0.951x+0.021 and good results were obtained (Fig 1).
2. The correlation in the high treatment area (TT15 % or less) also gave correlation coefficient of r=0.985, and regression formula of y=0.911x+0.209 and good results were obtained (Fig 2).
3. In the examination in 4 Lot PT reagent, CV of active % and the INR value were low, except CV of coagulation time (Table 2). On the expression of second, fluctuating range increased with the increase of INR (Fig 3).

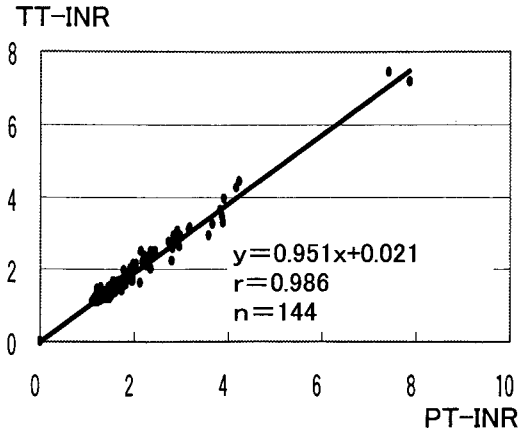

Fig. 1 The correlation of PT-INR and TT-INR in the whole treatment Area.

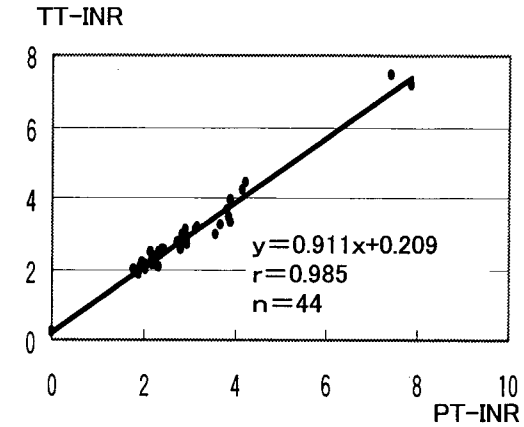

Fig. 2 The correlation of PT-INR and TT-INR in the high treatment area.

Table 2 The CV value of 4 Lot prothrombin reagents

	A	B	C	D
INR	2.1	3.6	3.8	5.5
active%	2.6	2.4	4.5	4.7
sec	2.2	4.4	8.1	10

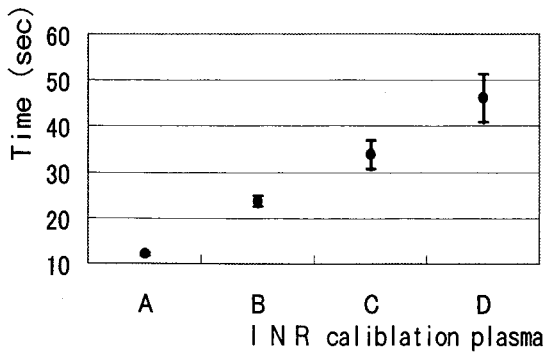

Fig. 3 Lot difference of the prothrombin reagents by the expression of seconds.

Ⅵ. Discussion

From the results of numerous previous survey of PT and TT [2,3,4,], it has been indicated that interlaboratory differences are due to sensitivity of the reagent and differences of the animal species, as well as differences of the analyzer in principle and the setting of the MNPT.

In the original method, MNPT uses the mean values of individual PT coagulation time of 20 healthy subjects (10 men and 10 women)[5]. However, it is difficult to actually make the reagent and use as an original routine test in practice.

In this country, there are many laboratories which use TT for the monitoring of oral anticoagulant therapy, and facilities reporting TT-INR test results are very few [3].

Although it was reported that the correlation worsened with PT of 8 % or less [2] in the active % display of TT, in the present results of INR display, the good correlations between TT-INR and PT-INR were obtained in both the high treatment area (TT15% or less) and the whole treatment area. Therefore, giving INR report by using calibration plasma for the monitoring of oral anticoagulant therapy is the most suitable and practical method for the standardization of TT as well as PT.

In addition, because MNPT can be calculated simply without requiring the standard blood plasma and the use of the INR calibration plasma is convenient, thus it is concluded that the method has a key merit of the routine test.

Moreover, on reactivity of the reagent and selection of the laboratory analyzer, integration is difficult in the competition in individual preference and specificity and between marketing products, and the only mean of improvement seems to be selection by the clinical and laboratory side.

It is a known fact that the compatibility between laboratories of PT that is commonly used for clotting test is defective in giving reports with second, time ratio and active %, apart from the INR system. The reality is that the area of disease distinction of congenital deficiency is judged by clinicians based on the experience at the facilities.

Also, the following are used in severe liver functional impairment and definitions of disseminated intravascular coagulation syndrome (DIC) and diagnostic criteria, etc. : second number, active %, title of the time ratio of PT. Recently, there are high hopes for using PT-INR for the diagnosis of DIC [2] because the momentum for reviewing DIC diagnostic criteria have been increasing both at home and abroad [6].

The measurement results showed that INR calibration plasma (A, B, C, D) measured using 4 Lots of PT reagent with lengthening coagulation time tended to widen, and the width of maximum value and minimum value, when the coagulation time was lengthened. This is a result that has been pointed out in the coagulation time (unit : second) report and the result shows that it can not be improved, even by using the INR calibration plasma. However, for standardization, the PT-INR report should be spread even in the filed of oral anticoagulant therapy.

It is important for the surveyor to make an effort to choose a reagent with small lot differences when making reports with second, time ratio, active % in actuality.

The results of our experiment are concluded that for standardization of PT with respect to differences of various reagents, measuring devices and test methods, the best method seems to use calibration plasma for monitoring. Yamada et al [7] have reported that INR calibration plasmas of 3 companies on the market had good results.

However, the guarantee of the accuracy of INR of each product has not been achieved, since the international regulation for the setting of INR value of the INR standard plasma has not been formulated. International standards of the calibration procedure are necessary in setting of INR display in the future.

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