The progress of prothrombin time measurement

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Abstract

Warfarin is the most widely used medicine for oral anticoagulant therapy (OAT). It inhibits the synthesis of coagulation factors II, VII, IX, and X in the liver and results in the production of inactive or partially active versions of these factors. Inactive coagulation factors interfere with prothrombin time measurement (Quick and Owren PT) measuring the sum of coagulation activity and inhibition. The narrow therapeutic range here involves a danger of serious complications and the risk of bleeding or thrombosis. The new-generation PT method can measure coagulation activity and inhibition separately. This new technique promotes patient care and anticoagulant medication (warfarin, dicoumarol) based on coagulation activity *in vivo*. Both therapy and laboratory controls should be unquestionably accurate and based solely on *in vivo* coagulation activity. Inactive coagulation factors (inhibition) render measurement, calibration, and harmonization. The use of the new-generation PT method based on measurement of coagulation activity *in vivo* could develop vitamin K antagonist (VKA) therapy for the marked benefit of patients.

History

Discovery of vitamin K antagonists (VKAs) in anticoagulation occurred in the 1920s, when the veterinarian Frank Schofield studied the hemorrhagic disease affecting cattle consuming sweet clover. He ascribed the bleeding to a toxin in the clover.[1] Karl Link and his team studied spoiled sweet clover and, in 1939, extracted dicumarol and identified the hemorrhagic agent, which was the substance in the sweet clover affecting coagulation.[2] The discovery of dicoumarol made it possible to inhibit thrombosis and to study anticoagulation therapy for humans in the early 1940s.[3,4] In the decades 1930 and 1940 Professor Armand Quick developed a routine prothrombin time (PT) coagulation test, which preceded the use of VKAs.[5,6] This has served as a basis for oral anticoagulant therapy (OAT) monitoring from the onset. However this first drug for OAT subsequently was found to have drawbacks by reason of its long half-life. Karl Link[7] synthesized more than 150 anticoagulant compounds and found one particularly active molecule, which was named warfarin (a 4-hydroxy compound) after the patent holder, the Wisconsin Alumni Research Foundation. Professor Paul Owren found factor V and developed a new PT method[8] subsequent to the "Quick method" to overcome its drawbacks. The Owren reagent (combined thromboplastin reagent, Thrombotest) is used mainly in the Nordic countries, Benelux, and Japan. By reason of its different reagent composition, the Quick technique is sensitive to the coagulation factors fibrinogen, II, V, VII, and X, while the Owren technique is affected by deficiencies in factors II, VII, and X. The Quick method measures factor V and fibrinogen, which are not dependent on warfarin therapy, and this constitutes a drawback for OAT.

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Warfarin

The coumarins or VKAs have been the mainstay of OAT for over 50 years. Warfarin is the most widely used drug for OAT world-wide, and it has a predictable onset and duration of action and excellent bioavailability. The therapy is effective for a variety of clinical indications of anticoagulation. The dosage and the clinical response still vary markedly among patients, depending on genetic inheritance, age, and metabolism.

Warfarin is a racemic mixture of two optically active isomers, the R and S forms. It is rapidly absorbed from the gastrointestinal tract (within 90 min) and its half-life is 36-42 h. In the circulation warfarin is bound to plasma proteins (mainly albumin). It accumulates rapidly in the liver, where it is metabolized.[9] Warfarin interferes with the cyclic interconversion of vitamin K and its 2,3 epoxide (vitamin K epoxide) and thus is a VKA. Vitamin K is a cofactor in the carboxylation of vitamin K-dependent coagulation factors. VKA inhibits the synthesis of coagulation factors II, VII, IX, and X in the liver, and they remain partially inactive unless 9 to 13 of the amino terminal glutamate (Glu) residues are carboxylated to form the Ca^{2+}-binding γ-carboxyglutamate (Gla) residues (Figure 1).[9-11] This carboxylation step renders the coagulation factors functionally active: binding to Ca^{2+} and the phospholipid surface.[10] Therapeutic dosages of warfarin decrease, by 30-50%, the total amount of each vitamin K-dependent coagulation factor synthesized by the liver. The secreted molecules from the liver are under-carboxylated, resulting in diminished biological activity (10-40% of normal).[11]

Warfarin treatment reduces the number of Gla residues (normal, 9 to 13) per clotting factor molecule, with a concomitant fall in coagulant activity. When the number of residues decreases from 13 to 9, only 70% of the activity of the clotting factor remains; when one molecule contains six carboxylated residues, only 2% activity is present.[12] The liver excretes both active and inactive coagulation factors to the plasma and both affect International Normalized Ratio (INR) measurement,[13] which possibly has escaped notice in the World Health Organization (WHO) recommendation for the prothrombin time methodology.

International Normalized Ratio

The calculation formula is for: INR = $(sample_{sec}/normal_{sec})^{ISI}$, where ISI is the International Sensitivity Index. When the ISI is near 1.0, the reagent is sensitive and ISI has little meaning in the INR calculation.

The Quick and Owren PT methods are the most common and generally accepted means of monitoring VKA therapy. A comparison between the PT methods has been published in a recent article[14] and the Owren PT was superior. WHO recommends the use of the INR to harmonize PT results and therapeutic ranges globally both for patient care in clinical practice and in the scientific literature, as the units used formerly proved inadequate for international communication.[15,16] The challenge is for global clinical laboratories to harmonize INR testing further to a level where INR results are consistent regardless of the methods used.

The use of the INR system still involves difficulties with sample citrate concentration,[17,18] different reagents and thromboplastins,[19] and instruments,[20,21] and with ISI and "local ISI" calibration[22-28] to harmonize results. Horsti and colleagues measured 150 samples from patients on oral anticoagulation using seven commercial reagents and four different calibrator kits. Agreement between INR results was poor.[29]

ISI calibration

The reagent manufacturer informs the ISI value or the laboratory can measure the ISI with an ISI calibration kit (local calibration) for a PT reagent lot. The meaning of ISI as power in the INR calculation equation is displayed in the previous section.

First, the aim in the original recommendation[16,30] was to harmonize INR results by calibrating reagent ISI values with Human Combined, which was the primary reference preparation (IRP code 67/40). Thromboplastins from different sources (human brain, rabbit brain, rabbit lung, and ox brain) yield quite different levels of PT. The hierarchy of reference thromboplastin preparations was presented by van den Besselaar and associates.[31] As the WHO calibration procedure was complex and demanding in the second stage, the recommendation for ISI calibration was local calibration using certified lyophilized plasmas.[32-37] A third possible approach for calibration was presented by van den Besselaar and group. This alternative means of determining the ISI involves the use of freshly pooled plasmas from 20 normal individuals and 60 patients receiving coumarin (OAT). Such numbers of samples are necessary to obtain a precise calibration line for ISI calculation. Freshly pooled plasma can be used to determine reagent or instrument ISI with acceptable precision, or as good a result as with the WHO calibration model.[38]

Poller and colleagues (European Concerted Action on Anticoagulation, ECAA) have compared local ISI calibration and "Direct INR" in the correction for locally reported INRs. For local INR correction (harmonization) they used seven normal plasmas and 20 from patients on warfarin. The results were variable.[39] It is clear that plasmas from patients on warfarin therapy always involve varying amounts of inactive coagulation factors, depending on the level of anticoagulation and individual patient characteristics (see section on Warfarin).

All these former calibration models involve the principle that the calibrator contains an average amount of inactive coagulation factors (inhibition), which means an average correction for patient INR results. Is the average correction of inactive coagulation factors appropriate for individual patient samples in a measuring range from 1 to 5 INR? Is it a fact that calibrators, according to biochemical principles, contain inactive coagulation factors (inhibition)? Do different reagents behave identically to active and inactive coagulation factors?[29] In our earlier studies we measured inactive coagulation factors (inhibition) in four different kits and noted conspicuous variation in inhibition.[40,41] ISI calibration should be based on normal plasma and normal plasma dilutions[42] without inactive coagulation factors. In principle, calibrators should not contain inhibitory coagulation factors, which cause error in calibration.[40]

The new-generation prothrombin time method

Today the Quick and Owren PT methods measure the sum of active coagulation factors (FII, FVII, FX) and inhibition by their inactive coagulation factor counterparts. The new-generation PT method can measure separately both active coagulation factors (FII, FVII, FX) and the inhibition caused by inactive or partially inactive coagulation factors.[40] Inhibition varies markedly between individual patient samples, depending on the medication and metabolism. Correction of inhibition must be made individually for each patient sample.[40] In this method two measurements for one sample are needed and the cost per sample is twice as much. The average correction does not suffice for accurate INR results. In addition, the number of inactive coagulation factors varies markedly in different calibrator kits (manufactured or local). This means difficulties in calibration and harmonization of different reagents.[29,41] The harmonization of INR results using different PT methods and reagents is problematic if attempted according to the WHO recommendation.[29,43] Measuring only the active coagulation factors without inhibition harmonization between different reagents succeeds well.[41]

Conclusions

New medications for OAT have been developed over a number of years and anticipated

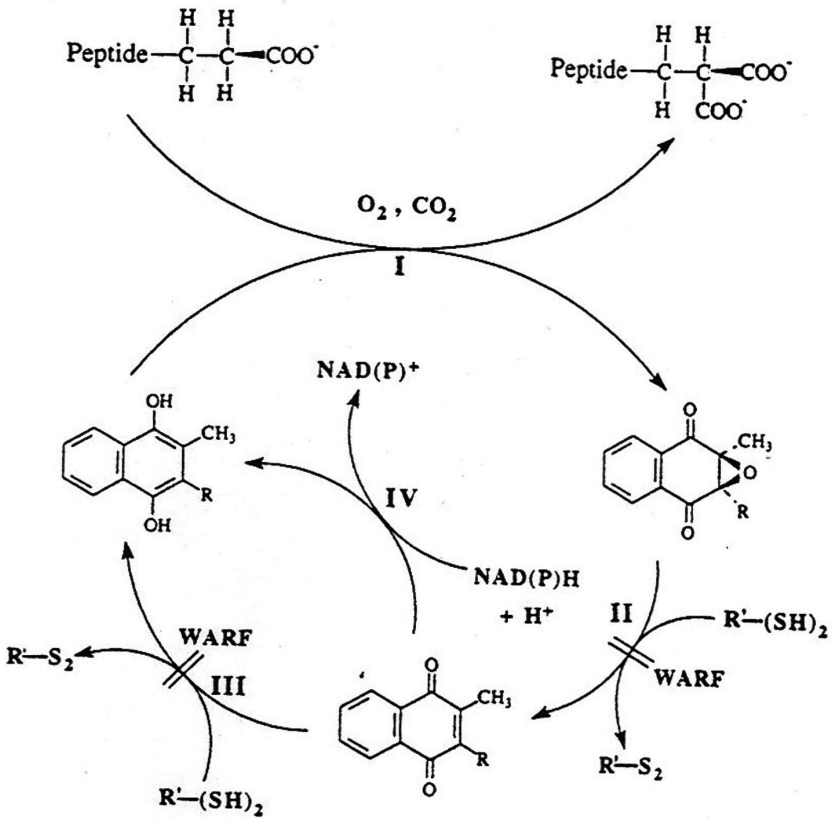

Figure 1. The enzyme reactions involved in the metabolic function of vitamin K. Vitamin K-dependent carboxylase catalyzes the transformation of peptide- (Factors II, VII, IX, and X) bound glutamate residues (Glu) to γ-carboxyglutamate (Gla) residues in the presence of vitamin K hydroquinone, carbon dioxide, and molecular oxygen (I). Vitamin K hydroquinone is oxidized in the reaction to vitamin K 2,3 epoxide. The reduction of the latter to vitamin K quinone is catalyzed by vitamin K epoxide reductase, which can use certain dithiols as the reductants (II). Vitamin K quinone can be reduced to vitamin K hydroquinone in the reactions catalyzed by either a dithiol-dependent (III) or an NAD(H)P-dependent (IV) entzyme. Warfarin (WARF) blocks reactions II and III. (Uotila L. Recent findings on the functions and requirements of vitamin K in humans. Klinlab 1998;3:97-101.)

without laboratory test control in an effort to displace warfarin medication. The new medicines, however, have not proved their superiority over warfarin. The new molecules are too expensive for global use or involve serious side-effects and possibly will never be as popular as warfarin, which is an old and cheap means widely used and accepted globally. The only drawback with warfarin medication is regular laboratory control. It would be important to develop warfarin therapy with greater attention to laboratory control, which helps patient care. Different reagents and thromboplastins react variably with inactive coagulation factors and cause difficulties in calibration using patient plasmas that contain inhibiting coagulation factors. Data on inactive coagulation factors are individual and should be corrected individually. Every new anticoagulant therapy patient should be tested for inhibition at the commencement of therapy.

The accuracy and harmonization of patient INR results for different reagents is of the utmost importance for scientific publications, medication, and patient care. Today's measuring principle is taking the sum of active coagulation factors (FII, FVII, FX), and inhibition of inactive coagulation factors is not a satisfactory approach from the standpoint of accurate patient care. Active coagulation factors *in vivo* are responsible for thrombosis and bleeding. The errors in INRs are too great, which in many ways affects the success of medication and patient well-being, also using the same calibration (local calibration).[29] The new-generation PT offers possibilities of more accurate INR results and patient care based on control of active coagulation factors. The inhibition renders measurement, calibration, and harmonization.[29,40,41] The therapeutic INR ranges guiding anticoagulant therapy using "old methods" are based on the principle that both calibrators and patient samples have inactive coagulation factors (inhibition) on average, which compensate for each other in the final INR result. Thus the therapeutic ranges are available for the new-generation PT method. The therapeutic range lies between thrombosis and bleeding, and complications are serious and general.[44] The medication and care of OAT patients must be rendered better and safe using a more sensitive PT test.

References

1. Schofield FW. Damaged sweet clover: the cause of a new disease in cattle simulating hemorrhagic septicemia and blackleg. J Am Vet Med Assoc 1924;64:553-75.
2. Link KP. The anticoagulant from spoiled sweet clover hay. Harvey Lect 1943-4;39:162-216.
3. Butt HR, Allen EU, Bollman JL. A preparation from spoiled sweet clover C, 3 methylene-bis-(4 hydroxy-coumarin) which prolongs coagulation and prothrombin time of blood: preliminary report of experiments and clinical studies. Mayo Clin Proc 1941;16:388.
4. Townsend SR, Mills ES. Effect of the synthetic haemorrhragic agent 4-hydroxycoumarin in prolonging the coagulation and prothrombin time. Can Med Assoc J 1942;46:214.
5. Quick AJ, Stanley-Brown M, Bancroft FW. A study of the coagulation defect in hemophilia and in jaundice. Am J Med Sci 1935; 190:501-11.
6. Quick AJ. The prothrombin time in hemophilia and in obstructive jaundice. J Biol Chem 1935;109:73-4.
7. Link KP. The discovery of dicoumarol and its sequels. Circulation 1959;9:97-107.
8. Owren PA. Thrombotest. A new method for controlling anticoagulant therapy. Lancet 1959;2:754-8.
9. Ansell J, Hirsh J, Poller L, et al. The pharmacology and management of the vitamin K antagonists: the seventh ACCP Conference on Antithrombotic and Thrombolytic Therapy. Chest 2004;126: 204-233.
10. Hirsh J, Fuster V, Ansell J, et al. American Heart Association/American College of Cardiology Foundation Guide to Warfarin Therapy. Circulation 2003;107:1692-734.
11. Majerus PW, Tollefsen DM. In: Goodman and Gilman's Pharmacological Basis of Therapeutics. 11th ed. Brunton LL, Lazo JS, Parker KL, ed. New York: McGraw-Hill, 2006, pp 1475-86.
12. Goodnight SH Jr, Hathaway WE. Disorders of Hemostasis and Thrombosis: a clinical guide. 2nd edn. New York: McGraw-Hill, 2001, p 554.
13. Suttie JW. Synthesis of vitamin K-dependent proteins. FASEB J 1993;7:445-52.
14. Horsti J. Has the Quick or the Owren prothrombin time method the advantage in harmonization for the International Normalized Ratio system? Blood Coag Fibrinolysis 2002; 13:641-6.
15. International Committee for Standardi sation in Haematology. International Committee on Thrombosis and Haemostastis. ICSH/ICTH recommendations for reporting prothrombin time in oral anticoagulant control. Thromb Haemost 1985;53:155-6.
16. WHO Expert Committee on Biological Standardisation. Thirty-third Report. Technical Report Series 687. WHO Geneva 1983;81-105.
17. Horsti J. Preanalytical aspects of routine coagulation measurements. Scand J Clin Lab Invest 2001;61:167-8.
18. Adcock DM, Kressin DC, Marlar RA. Effect of 3.2% vs. 3.8 % sodium citrate concentration on routine coagulation testing. Am J Clin Pathol 1997;107:105-110.
19. van den Besselaar AM. International standardisation of laboratory control of oral anticoagulant therapy: a survey of thromboplastin reagents used for prothrombin time testing. J Heart Valve Dis 1993;2:42-52.
20. Chantarangkul V, Tripoldi A, Clerici M, et al. Assessment of the influence of citrate concentration on the International Normalised Ratio (INR) determined with twelve reagent-instrument combinations. Thromb Haemost 1998;80:258-62.
21. Cunningham MT, Johnson GF, Pennell BJ, et al. The reliability of manufacturer-determined, instrument-specific international sensitivity index values for calculating the international normalised ratio. Am J Clin Pathol 1994;102:128-33.
22. van Rijn JL, Schmidt NA, Rutten WP. Correction of instrument- and reagent-based differences in determination of the International Normalised Ratio (INR) for monitoring anticoagulant therapy. Clin Chem 1989;35:840-3.
23. Ng VL, Levin J, Corash L, et al. Correction of instrument- and reagent-based differences in determination of the International Normalised Ratio (INR) for monitoring anticoagulant therapy. Am J Clin Pathol 1993;99:689.
24. Talstad I. Why Is the standardisation of prothrombin time a problem? Haemostasis 2000;30:258-67.
25. Kirkwood TB. Calibration of reference thromboplastins and standardisation of the prothrombin time ratio. Thromb Haemost 1983;49:238-44.
26. Hermans J, van den Besselaar AM, Loeliger EA, et al. A collaborative calibration study of reference materials for thromboplastins. Thromb Haemost 1983;50:712-7.
27. Craig S, Stevenson KJ, Dufty JM, et al. Local INR correction: justification for a simplified approach. J Clin Pathol 1997;50: 783-9.
28. Stevenson KJ, Craig S, Dufty JM, et al. System ISI calibration: a universally applicable scheme is possible only when coumarin plasma calibrants are used. Br J Haematol 1997;96:435-41.
29. Horsti J, Uppa H, Vilpo J. Poor agreement between different prothrombin time International Normalized Ratio (INR) methods: comparison of seven commercial reagents. Clin Chem 2005;51:553-60.
30. Hermans J, van den Besselaar AMHP, Loeliger EA, et al. A collaborative calibration study of reference materials for thromboplastins. Thromb Haemost 1983;

50:712-7.
31. van den Besselaar AMHP, Lewis SM, Mannucci PM. Status of present and candidate international reference preparations (IRP) of thromboplastin for the prothrombin time: a report of the Subcommittee for Control of Anticoagulation (Letter). Thromb Haemost 1993;69:85.
32. Clarke K, Taberner DA, Thomson JM, et al. Assessment of value of calibrated lyophilised plasmas to determine International Sensitivity Index for coagulometers. J Clin J Clin Pathol 1992;45:58-60.
33. Poller L, Triplett DA, Hirsh J, et al. Assessment of value of calibrated lyophilised plasmas to determine International Sensitivity Index for coagulometers. Am J Clin J Clin Pathol 1995; 103:358-65.
34. Poller L, Barrowcliffe TW, van den Besselaar AMHP, et al. European Concerted Action on Anticoagulation. A simplified statistical method for local INR using linear regression. Br J Haematol 1997; 98:640-7.
35 Poller L, Barrowcliffe TW, van den Besselaar AM, et al. European Concerted Action on Anticoagulation (ECAA). Minimum lyophilised plasma requirement for ISI calibration. Am J Clin Pathol 1998; 109:196-204.
36. Poller L, van den Besselaar AMHP, Jespersen J, et al. A comparison of linear and orthogonal regression analysis for local INR determination in ECAA coagulometer studies. Br J Haematol 1998;102: 910-7.
37. Plesch W, van den Besselaar AM. Validation of the International Normalized Ratio (INR) in a new point-of-care system designed for home monitoring of oral anticoagulation therapy. Int J Lab Hematol 2009;31:20-5.
38. van den Besselaar AM, Witteveen E, Schaefer-van Mansfeld H, et al. Effect of plasma pooling on the International Sensitivity Index of prothrombin time systems. Blood Coagul Fibrinolysis 1998; 9:645-51.
39. Poller L, Keown M, Ibrahim S, et al. European Concerted Action on Anticoagulation. Comparison of local International Sensitivity Index calibration and "Direct INR" methods on correction of locally reported International Normalized Ratios: an international study. J Thromb Haemost 2007;5:1002-9.
40. Horsti J, Uppa H, Vilpo JA. A new generation prothrombin time method for INR. Open Med Chem J 2008;2:11-5.
41. Horsti J, Uppa H, Vilpo JA. A new-generation method for Quick and owren PT. Open Hematol J 2008;2:81-5.
42. Lindahl TL, Egberg N, Hillarp A, et al. INR calibration of Owren-type prothrombin time based on the relationship between PT% and INR utilizing normal plasma samples. Thromb Haemost 2004;91:1223-31.
43. Horsti J. Comparison of Quick and Owren prothrombin time with regard to the harmonisation of the international normalised ration (INR) system. Clin Chem Lab Med 2002;40:399-403.
44. Odén A, Fahlén M. Oral anticoagulation and risk of death: a medical record linkage study. Br Med J 2002;325:1073-5.