Effect of Phenprocoumon on Monitoring of Lepirudin, Argatroban, Melagatran and Unfractionated Heparin with the PiCT Method

T. Fenyvesi I. Jörg J. Harenberg

Fourth Medical Department, University Hospital, Mannheim, Germany

Key Words

Prothrombinase-induced clotting time · Thrombin inhibitors · Phenprocoumon · Additive effects

Abstract

Prothrombinase-induced clotting time (PiCT) is a clotting-time test for heparins and direct thrombin inhibitors to reduce drawbacks of aPTT. Effects of the direct thrombin inhibitors lepirudin, argatroban, melagatran and of unfractionated heparin (UFH) were investigated in normal and oral anticoagulant plasma samples. Lepirudin showed potentiating interferences with phenprocoumon effects. Melagatran, argatroban and UFH delivered distinct linear additive effects in both plasma sample groups. PiCT ratio reduces differences between both groups with UFH, and argatroban inhibitor-receptor-binding mode plays a role in interaction patterns.

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Introduction

In clinical use, heparins and direct thrombin inhibitors are mostly monitored by aPTT [1, 2]. Limitations of aPTT methods include non-linear dose-effect relationships with a plateau effect, variability between different testing instruments, reagents and different lots of the same reagent [3]. These limitations are enhanced when patients receive an overlapping therapy consisting of an oral anticoagulant in combination with heparin or a direct thrombin inhibitor.

Recently, a snake venom-based testing method, the ecarin clotting time (ECT), has been developed to obtain linear dose-response relationships with direct thrombin inhibitors. This method is therefore more accurate in monitoring hirudin and argatroban than aPTT [2, 4]. However, ECT is insensitive to heparins because heparins require antithrombin, which cannot react with meizothrombin or other intermediates of the prothrombin-thrombin conversion with a thrombin activity [e.g. meizothrombin (desF1)] [5]. Prothrombinase-induced clotting time (PiCT) [6] is another novel testing technique based on clot activation by a toxin of a snake venom. RVV-Va from Russel's viper venom in the reagent activates factor (F) V present in the test set-up. FVa forms the

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1424–8832/02/0324–0174\$18.50/0

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J. Harenberg
Fourth Medical Department, University Hospital
Theodor-Kutzer-Ufer 1–3
D–68167 Mannheim (Germany)
Tel. +49 621 383 3378, Fax +49 621 383 3808, E-Mail j-harenberg@t-online.de

prothrombinase complex with FXa (added in reagent), phospholipids and calcium ions. PiCT determines activities of direct and indirect thrombin inhibitors in a linear manner over a wide concentration range (up to 2,000 ng/ml) [6]. As PiCT is based on factors affected by oral anticoagulation (FX and FII), this test is presumably also sensitive to oral anticoagulants, just as aPTT and PT are [7–10], due to lower levels of these factors during therapy with vitamin K antagonists.

In the present study, we describe the effects of three direct thrombin inhibitors (hirudin, argatroban and melagatran) and of unfractionated heparin (UFH) on PiCT in normal plasma and in plasma of patients on steady-state oral anticoagulation with phenprocoumon. Oral anticoagulation may be switched to direct thrombin inhibitors or heparins in special clinical settings like invasive diagnostics and surgery. During overlapping therapy, vitamin K antagonists as well as thrombin inhibitors are present and may have additive effects. The aim of the study was to determine the influence of oral anticoagulants on the monitoring of the aforementioned drugs and to investigate the utility of the method for monitoring UFH and direct thrombin inhibitors during oral anticoagulation.

Materials and Methods

Blood samples (~ 10 ml) of 4 healthy volunteers (normal plasma, NP) and 6 patients (INR: 2.21 ± 0.31 , mean $\pm$ SD) on treatment (oral anticoagulant plasma, OACP) with the vitamin K antagonist phenprocoumon (Hoffmann-La Roche, Basel, Switzerland) were collected by clean cubital vein puncture into plastic vials with sodium citrate (3.8%; plasma/citrate: 9/1, V/V) and kaolin (coagulation time 30 min). The centrifuged plasma samples (1,800 g for 10 min) were either tested immediately or shock frozen in liquid nitrogen and stored at -80°C for analyses within 4 weeks. Plasma samples were spiked with concentrations ranging from 300 to 3,000 ng/ml of r-hirudin (Lepirudin®, Aventis, Frankfurt/Main, Germany), argatroban (by courtesy of Mitsubishi Chemical Corp., Tokyo, Japan), and UFH (Liquemin®, Hoffmann-La Roche) or melagatran ranging from 30 to 1,000 ng/ml (kindly provided by AstraZeneca, Mölndal, Sweden). In the case of heparin, 300 and 3,000 ng/ml corresponded to 0.048 IU/ml and 0.48 IU/ml, based on a content of 160 IU/mg dry substance and an average molecular mass of 12.5 kD. All PiCT measurements were carried out in a KC 10a micro® device from Amelung Comp. (Lemgo, Germany) [11]. The prothrombinase reagent (test lot #1) containing FV activator (RVV-FVa from Russel's viper venom), FXa and phospholipids was kindly provided by Pentapharm Ltd. (Basel, Switzerland). All data are given as mean values with standard deviations. For all calculations the mathematical and statistical functions of Microsoft Excel were used.

Results

PiCT Expressed in Seconds

Normal PiCT range was 29.9 ± 4.6 in NP. In OACP, this value was prolonged to 36.3 ± 20.3 s. Lepirudin at 3,000 ng/ml delivered PiCT values of 128.0 ± 23.4 s in NP and over 600 s in OACP (fig. 1a). The same amount of argatroban led to PiCT values of 151.0 ± 23.9 s in NP and 233.7 ± 57.2 s in OACP samples (fig. 1b). Maximum dose of melagatran (1,000 ng/ml) caused clotting times of 153.5 ± 9.9 s in NP and 342.6 ± 78.0 s in OACP (fig. 1c). By spiking with 3,000 ng/ml (0.48 IU/ml) UFH, PiCT prolongations to 137.0 ± 8.4 s could be obtained with NP. In OACP, the presence of 3,000 ng/ml (0.48 IU/ml) UFH prolonged aPTT to 186.8 ± 42.2 s (fig. 1d). PiCT provided good linearity with UFH throughout the entire concentration range in NP as well as in OACP. Argatroban and melagatran also showed fairly good linearity in both groups for most of the concentration ranges tested. Lepirudin delivered a flat linear dose-response curve with NP up to 3,000 ng/ml and in OACP up to 2,000 ng/ml.

Individual Normalized PiCT Ratios

The question emerged whether differences between NP and OACP samples could be substantially reduced or even abolished by formulating a ratio. With lepirudin, the maximal dose of 3,000 ng/ml provided normalized PiCT values of 4.28 ± 0.18 in NP (fig. 2a). In OACP, the same concentration delivered a ratio of 16.6 (SD not determined). With argatroban, the normalized ratio almost abolished the differences between both groups (fig. 2b). The maximum concentration tested (3,000 ng/ml) provided ratios of 5.0 ± 0.16 versus 6.44 ± 0.2 in NP and OACP, respectively. With melagatran, PiCT ratios of 5.13 ± 0.06 versus 9.4 ± 0.23 were calculated when adding 1,000 ng/ml to NP and OACP, respectively (fig. 2c). A good equalising effect was also found with UFH, especially in higher concentration ranges (fig. 2d). Adding 3,000 ng/ml (0.48 IU/ml) UFH delivered ratios around 5 in both groups (4.58 ± 0.06 in NP versus 5.15 ± 0.23 in OACP).

Suggested Therapeutic Ranges for PiCT

Based on the results presented here, therapeutic ranges can be suggested for direct thrombin inhibitors and UFH in normal plasma and during oral anticoagulation. PiCT has a more pronounced concentration-effect relationship with heparin and direct thrombin inhibitors than aPTT. With aPTT, the therapeutic range is a 1.5- to 3-fold prolongation of clotting times [12, 13]. Therefore, a higher

Fig. 1. Concentration/activity-PiCT relationships of hirudin (a), argatroban (b), melagatran (c) and UFH (d) in normal plasma (NP; $n = 4$) and plasma samples of patients on oral anticoagulation with phenprocoumon (OACP; $n = 6$). Data are means $\pm$ SD.

range of PiCT ratios, namely 2–3.5, corresponds to concentration ranges which would also be achieved during therapy with UFH [12, aPTT monitoring], r-hirudin [14, 15, ECT monitoring] or argatroban [9, ECT monitoring]. Clinical data for PiCT monitoring are not available yet. Based on these ratios, the following therapeutic concentration ranges were calculated from the concentration-PiCT ratio relationships: between 600 (0.096 IU/ml) and 1,550 ng/ml (0.248 IU/ml) for UFH in NP and OACP. With lepirudin, 1,000–2,600 ng/ml were calculated for NP and 650–1,350 ng/ml for OACP. In the case of argatroban, therapeutic ranges of 225–1,200 ng/ml in NP and 225–1,000 ng/ml in OACP were found. Therapeutic concentration ranges for melagatran were 30–435 ng/ml in NP and 30–105 ng/ml in OACP.

Based on the normal PiCT range of 29.9 ± 4.6 s in NP, the therapeutic range should be from 60 s (50–70 s; 59.8 ± 9.2) to 105 (up to 120 s; 104.7 ± 16.1).

Discussion

Differences of PiCT (in seconds) between NP samples and those on stable vitamin K antagonism therapy with phenprocoumon were found with all four drugs tested. By formulating individual normalized ratios, these differences could be abolished in the case of UFH and reduced in the case of argatroban and melagatran. With lepirudin, a weaker reduction effect was achieved, especially in higher concentration ranges.

The influence of oral anticoagulation on PiCT monitoring showed different patterns in the drugs tested. When testing UFH, interferences of linear additive appearance occurred only in medium and high concentration ranges (1,000–3,000 ng/ml). However, no interferences appeared below 1,000 ng/ml. In the case of lepirudin, interferences between NP and OACP were potentiating with higher concentrations, but little effects occurred in the lower concentration range. This could possibly indicate a hyperad-

Fig. 2. Concentration-normalized PiCT ratio relationships of hirudin (a), argatroban (b), melagatran (c) and UFH (d) in normal plasma (NP; n = 4) and plasma samples of patients on oral anticoagulation with phenprocoumon (OA; n = 6). Data are means $\pm$ SD.

ditive effect in the presence of higher lepirudin concentrations. In contrast to lepirudin, the two other direct thrombin inhibitors, argatroban and melagatran, showed a linear additive influence on oral anticoagulation throughout the concentration range. No hyperadditive phenomenon occurred at the upper ends of the concentration-effect curves, in contrast to lepirudin.

The differing patterns of interactions with oral anticoagulant effects between UFH on the one hand and direct thrombin inhibitors on the other could possibly be explained by their differing modes of action. The tripeptidic inhibitors argatroban and melagatran act with a one-to-one stoichiometry by monovalent binding to the catalytic active site of thrombin [16]. In the case of UFH and lepirudin, modes of action are more complex. UFH requires antithrombin as a cofactor for inhibition of thrombin, whereas lepirudin is a bivalent ligand for both the catalytic active site and the anion-binding exosite of thrombin [17, 18]. In the case of lepirudin a more complex stoi-

chiometry mediated by two binding sites leads to sigmoidal concentration-effect relationships. Besides different target sites, eventual affinity changes during oral anticoagulation due to higher relative inhibitor concentrations [19] might explain the diversity of interactions with oral anticoagulation of lepirudin versus argatroban and melagatran.

As a further possible mechanism, decreased carboxylation of coagulation factors II, VII, IX and X could alter the accessibility of the respective binding sites to different extents for each drug during therapy with vitamin K antagonists [20]. An eventual inhibition promiscuity for FII and FX described for some inhibitors [21] should also be taken into consideration as a possible reason. Feedback mechanisms between FII, FVII, FIX and FX could also play a role in generating the differences between the effects of oral anticoagulation on the actions of each direct thrombin inhibitor [22]. However, the precise mechanisms of these differences remain to be investigated.

A practical implication of dose-response curve results for the therapeutic concentration ranges, based on a PiCT prolongation of 2.0–3.5 of the normal value. Due to more pronounced dose-PiCT ratio relationships in OACP, dosages are to be reduced in OACP. As the initial parts of the curves showed no significant differences between UFH, argatroban and melagatran, lower boundaries of therapeutic concentration ranges are identical in both groups, only the upper boundaries are different for NP and OACP. Lepirudin showed significant differences also at low therapeutic concentrations, leading to different boundaries of therapeutic concentration ranges for both groups.

The results of the present work indicate that the sensitivity of the method is suitably high for monitoring (un-fractionated) heparins and direct thrombin inhibitors. The direct thrombin inhibitors hirudin and argatroban are established to maintain effective anticoagulation in patients with heparin-induced thrombocytopenia without (HIT type I) and with thrombosis (HITS or HIT type II) [23–25]. Melagatran is currently under investigation in clinical studies [26–29].

Calatzis et al. [6] reported a normal PiCT range up to 30 s. The normal PiCT range in the present study, $29.9 \pm$

4.6 s, agrees with their results. Their concentration-effect curves of hirudin (tested up to 2,500 ng/ml) and UFH [tested up to 1 IU/ml (6,250 ng/ml)] were similar to those found in the present work. However, the dose-effect relationship of lepirudin is not as pronounced with PiCT as with ECT [30, 31].

According to our results and those described by Calatzis et al. [6], PiCT seems to be a suitable method for clinical practice in supervising dosages of heparins (UFH and LMWH [6]) and the direct thrombin inhibitors hirudin, argatroban and melagatran. The latter, however, do not need monitoring in clinical practice, according to the specifications of the manufacturer, because of the good predictability of drug levels proven in clinical trials [26, 28, 32].

Acknowledgements

We would like to thank Mrs. Christina Giese, Mrs. Antje Hagedorn and Mrs. Inge Traeger for their excellent laboratory work and patient care. This study was supported by a grant of the Faculty of Clinical Medicine Mannheim, University of Heidelberg.

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