Here we describe the additive and synergistic actions of the DTIs lepirudin, argatroban, and melagatran on the effects of the oral anticoagulant phenprocoumon on PT. These synergisms interfere with the analysis and dose adjustment of oral anticoagulants during concomitant therapy periods. Data were derived from plasma from healthy volunteers and from patients treated with the vitamin K antagonist phenprocoumon.

Blood from 6 healthy volunteers and 10 patients undergoing treatment with the vitamin K antagonist phenprocoumon (Hoffmann-La Roche) was collected by clean cubital vein puncture into plastic vials containing 38 mL/L sodium citrate (9 mL of plasma in 1 mL of citrate). All donors (volunteers and patients) gave informed consent in accordance with the current revision of the Helsinki Declaration. After centrifugation (1800g for 10 min), plasma samples were shock frozen in liquid nitrogen, stored at −80 °C, and analyzed within 4 weeks. After thawing, plasma samples were supplemented with lepirudin (molecular mass ~6500 Da; obtained from Aventis) and argatroban (molecular mass 526.7 Da; kindly provided by Mitsubishi Chemical Corp., Tokyo, Japan) in concentrations ranging from 300 to 3000 μg/L. Melagatran (molecular mass 473.6 Da; courtesy of Astra Zeneca, Mölndal, Sweden) was added at lower concentrations, between 30 and 1000 μg/L, because of its higher gravimetric potency observed in preliminary experiments.

Clotting time measurements were carried out in a KC 10a microdevice (22) from Amelung Co. PT was determined with a recombinant thromboplatin reagent (Aventis Behring; lot number 526935; international sensitivity index = 1.09). With this thromboplatin reagent, clotting times can be determined up to ~600 s with this device. Interassay CVs were 10%, 8.7%, and 9.9% at clotting times of 10, 50, and 300 s, respectively (n = 12). To start the clotting time assay, 50 μL of plasma was incubated at 37 °C for 3 min (micromethod). To initiate clot formation, 100 μL of PT reagent (dissolved according to the manufacturer’s instructions) was added. The clotting times (PT) were transformed into INRs with an equation appropriate for the KC 10a device, obtained from the manufacturer of the thromboplatin reagent: PT (in seconds) × 0.09 + 0.2 = INR. The mean value obtained for the healthy volunteers was 10.2 ± 0.3 s, corresponding to an INR range of 1.1 ± 0.03.

All data are presented as the mean ± SD. Calculation factors (ng × factor = nmol/L) for transformation of the data from gravimetric scaling to an equimolar scale were 0.154 for lepirudin, 1.901 for argatroban, and 2.11 for melagatran, respectively. For reverse transformation, the factors were 6.5 for lepirudin, 0.526 for argatroban, and 0.474 for melagatran.

The PT was prolonged in OAC plasma (OACP; samples from patients undergoing a stable phase of OAC therapy) to 26.0 ± 5.0 s. All DTIs prolonged PT values in a concentration-dependent manner in plasma from the controls and patients receiving OAC therapy. The results are displayed in Fig. 1 in double scaling (nmol/L and μg/L). In Fig. 1, the upper therapeutic gravimetric concentrations
are compared in equimolar scaling. Lepirudin at 308 nmol/L (2000 μg/L) prolonged PTs to 12.4 ± 0.5 s and 70.3 ± 36.8 s in plasma samples from healthy volunteers (normal plasma) and OACP samples. Argatroban at 1901 nmol/L (1000 μg/L) yielded PTs of 25.7 ± 3.2 s in normal plasma and 94.5 ± 26.4 s in OACP, respectively. Melagatran at 633 nmol/L (300 μg/L) prolonged PTs to 16.8 ± 1.0 s in normal plasma and 117.8 ± 47.2 s in OACP. The INRs can be calculated with the equation given above.

OAC strongly influenced all concentration–PT relationships. The influence of PT reagents on the PT coagulation assay has been reported (16, 25). Here we describe the actions of various DTIs on the prothrombin coagulation assay with the use of a single thromboplastin reagent. The effects of argatroban and melagatran were influenced by OAC throughout the entire concentration range tested. In contrast, the influence of lepirudin on the PT was more strongly affected by OAC at higher concentrations [308 nmol/L (2000 μg/L)]. Up to this concentration, only a slight effect occurred. Lepirudin and hirulogs are directed against the active catalytic site as well as against the anion-binding exosite of thrombin (26, 27). Argatroban and melagatran are monovalent inhibitors of the catalytic active site of thrombin (28). The anion-binding exosite is responsible for the recognition of fibrinogen and may play an important role in mediating the different inhibition patterns of lepirudin compared with argatroban or melagatran. Recently, a novel oligopeptidic compound was described that specifically inhibits the anion-binding...
exosite. In a rat venous thrombosis model, its median effective dose was lower than that of recombinant hirudin or of argatroban (29).

In addition to differences regarding target sites and affinities of DTIs, the data presented here may also indicate some other interactions. These interferences could be mediated by other clotting factors affected by vitamin K antagonism. During OAC, in addition to decreased thrombin activity, concentrations of factors VII, IX, and X are decreased. Changes in the relationships of factor concentrations as a result of vitamin K antagonism could in turn alter feedback mechanisms between different factors of the coagulation cascade. Feedback mechanisms between thrombin and coagulation factors V, VII, and X are described in the literature (30). Finally, certain DTIs lack specificity for factor II and additionally inhibit factor X (31). Such a mechanism could partly explain the lack of uniformity of interactions between OACs and direct thrombin antagonists. Carboxylation of prothrombin may affect the conformation of the anionic binding site. Decreased carboxylation of factor II with OACs could in turn alter the accessibility of the binding site. Furthermore, the anion-binding exosite could be activated by a higher relative inhibitor concentration in OACP samples. Slow-binding inhibitor–receptor interactions can be potentiated by higher relative inhibitor concentrations with OACs (32). The precise mechanisms of the interactions remain, however, to be further investigated. A pharmacokinetic interference seems to be unlikely. OAC with warfarin does not alter the pharmacokinetics of napsagatan, but strongly affects its pharmacodynamics (33).

On the basis of our results and the interpretation described, it seems difficult to predict to what extent PTs or INRs are affected during concomitant therapy. Models described in the literature deal with a single DTI (argatroban or lepirudin) combined with either warfarin or phenprocoumon (15, 16, 18, 34). When the same DTI is used, results are similar with both vitamin K antagonists because the actions of phenprocoumon and warfarin on clotting times are mediated by comparable lowering of clotting factor concentrations (unpublished results). In contrast, each competitive thrombin inhibitor has its own kinetics at its binding site(s). Upper therapeutic limits are ~308 nmol/L (2000 µg/L) for lepirudin, 633 nmol/L (300 µg/L) for melagatran, and 1901 nmol/L (1000 µg/L) for argatroban. Our results demonstrate considerable differences between the effects of DTIs on PT values. These differences are even more pronounced in plasma from patients during stable OAC therapy.

Considering these differences, it might be difficult to establish a single model for all DTIs to predict PT (INR) prolongations during concomitant application periods. Simultaneous measurements of PT and of the concentrations of the DTIs by specific coagulation testing methods (e.g., ecarin clotting time), chromogenic (S-2238), ELISA, or chromatographic assays may improve judgment about the correct dosing during periods of concomitant therapy with vitamin K antagonists and DTIs. At present, when patients are switched from heparins and low-molecular-weight heparins to OAC, the current American College of Chest Physicians guidelines recommend discontinuation of heparins when the INR is in the therapeutic range (2, 3) for 2 consecutive days (35). However, the effects of heparins can be antagonized by adding protamine or heparinase to PT reagents, as mentioned above, although no antagonists are yet available for DTIs. On the basis of the data presented, more accurately detailed dose adjustment regimens may be required for concomitant treatment with vitamin K antagonists and each DTI.

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References

Acetylcholinesterase Activity and Biogenic Amines in Phenylketonuria, Kleopatra H. Schulpis,
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Phenylketonuria (PKU) is a disorder in which the aromatic amino acid Phe cannot be converted to Tyr (1, 2). Unfortunately, many PKU patients do not adhere to their
low-Phe diet (off diet), which leads to high concentrations of the amino acid in their blood (1, 2). High Phe concentrations interfere with the production of adrenaline (A),
noradrenaline (NA), and dopamine (DA) (1, 3). Furthermore, Krause et al. (4) reported an inverse relationship between NA and DA plasma concentrations and Phe
because high Phe concentrations decrease the availability of the amino acids Tyr and Trp, the precursors of cat-
echolamines and serotonin [5-hydroxytryptamine (5HT)], respectively (5–7).

Acetylcholinesterase (AChE) is a membrane-bound enzyme with its active site exposed at the external leaflet of the bilayer (ectoenzyme). When the enzyme is inhibited, it
can no longer participate in the hydrolysis of acetylcholine (ACh) (8), involving parasympathetic, sympathetic, peripheral, and central nervous system function (8–10).
Alterations of the above substances in the cerebrospinal fluid are correlated with AChE activity in the cerebrospinal fluid of patients with mental impairment (11).

In our previous study (12), incubation of high Phe concentrations with human AChE type XIII led to inhibition of the enzyme (40–60%). The effect of Phe on AChE of rat diaphragm and rat brain showed a concentration-dependent enzyme inhibition (13, 14). We therefore
aimed to evaluate AChE activities in the erythrocyte membranes from patients with PKU and to correlate the enzyme activities with blood concentrations of the biogenic amines A, NA, DA, and 5HT as well as with the precursors Tyr and Trp.
The study was approved by the Greek ethics committee and was conducted according to the principles expressed in the Helsinki Declaration.

The study population consisted of 23 PKU patients who were divided into two groups: group A (n = 12; mean age, 6.8 ± 1.2 years), who adhered strictly to their special therapeutic diet as evidenced by their almost normal plasma Phe concentrations (Phe, 180.4 ± 30.7 μmol/L); and group B (n = 11; mean age, 7.2 ± 2.0 years), who were off diet and had increased Phe concentrations (Phe, 1722 ± 286 μmol/L). Twenty-three healthy children of comparable age were the controls. All PKU patients were admitted to the day clinic of the Inborn Errors of Metabolism Department of the Institute of Child Health in Athens.

All blood samples were collected from an antecubital vein at the same time of day while both patients and controls were at rest. Blood samples (7.0 mL) were collected 3 h after participants arrived at our hospital, during which time the children fasted and were acclimatized to the hospital environment and staff.

Venous blood samples were collected into heparin-containing blood collection tubes from PKU patients and controls. The washed erythrocytes were lysed, as described by Galbraith and Watts (15) and Kamber et al. (16), after being frozen (−80 °C) and thawed (50 °C) five times. Membranes were suspended in 0.1 mol/L Tris-
HCl, pH 7.4, to a final protein concentration of 2 g/L (17). The minor hemoglobin that remained attached to the membrane surface was measured by reagent set 527-A.