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Mini-Review

A review of the effects of the oral direct thrombin inhibitor ximelagatran on coagulation assays

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Introduction

Ximelagatran is the first oral agent in the new anticoagulant drug class of direct thrombin inhibitors (DTIs)[1] and has been clinically developed using fixed dosing without the use of coagulation monitoring. While coagulation monitoring is not required with ximelagatran, effects on various coagulation assays have been studied. This paper describes the effects of melagatran, the active form of ximelagatran, on different coagulation assays and considers their possible use in special clinical situations.

Ximelagatran has been evaluated in a range of potential indications, including the prevention of venous thromboembolism (VTE) in orthopaedic surgery patients [1–4], the prevention of stroke associated with atrial fibrillation [5–7], the treatment [8] and long-term secondary prevention [9] of VTE, and prevention of major cardiovascular events following acute myocardial infarction [10]. In all indications, fixed-dose regimens of ximelagatran have provided effective

Abbreviations: ACT, activated clotting time; APTT, activated partial thromboplastin time; DTI, direct thrombin inhibitor; ECT, ecarin clotting time; FV, factor V; IC_{50}, plasma concentration of anticoagulant agent that doubles the clotting time compared with anticoagulant-free plasma; INR, International Normalized Ratio; ISI, International Sensitivity Index; LC, liquid chromatography; LMWH, low-molecular-weight heparin; PiCT, prothrombinase-induced clotting time; PT, prothrombin time; PTC, prothrombin complex; TT, thrombin clotting time; UFH, unfractionated heparin; VTE, venous thromboembolism.

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anticoagulation combined with an acceptable bleeding profile, without the use of coagulation monitoring or dose adjustments.

Following oral administration, ximelagatran is rapidly absorbed and bioconverted to its active form melagatran via two intermediates (ethyl-melagatran and hydroxy-melagatran). Melagatran binds directly, reversibly, and with high affinity to the active site of thrombin, providing potent inhibition of thrombin activity in vitro (K_i=2 nmol/l) [11]. Dose-dependent inhibition of thrombin generation—apparently mediated, in part, by blockade of the feedback activation of factor V (FV) by thrombin [12]—as well as inhibition of platelet activation have also been demonstrated ex vivo following oral administration of ximelagatran to healthy volunteers [13,14]. The inhibition of thrombin activity, thrombin generation, and platelet activation by melagatran results in reduced thrombus formation ex vivo, with dose-dependent reductions in total thrombus area and total fibrin area formed on denuded pig aorta following oral administration of ximelagatran to healthy volunteers [15].

Peak plasma melagatran concentrations are reached approximately 2 h after oral dosing of ximelagatran [16]. Melagatran can also be administered subcutaneously. Low variability in melagatran pharmacokinetics has been demonstrated in young, healthy volunteers after either oral ximelagatran or subcutaneous melagatran administration [16,17]. The pharmacokinetic properties of melagatran in patients are comparable to those in young, healthy volunteers [18,19]. However, since systemic melagatran is primarily eliminated renally [20], the general decline in renal function with increasing age can lead to higher exposure to melagatran in these patients. When administered twice daily, the fluctuation between peak and trough plasma concentrations of melagatran is moderate, with approximately a threefold difference between the two [18,19]. There is a low potential for drug interactions mediated by the cytochrome *P450* system [21], and coadministration with food does not affect the pharmacokinetics of melagatran [16]. The stable and reproducible pharmacokinetic profile supports fixed-dose regimens without dose adjustment or coagulation monitoring.

Melagatran does affect the different coagulation assays, and so it is important to know what effects can be expected with each of the assays. The effects of melagatran on assays widely used in clinical practice [Activated Partial Thromboplastin Time (APTT), Activated Clotting Time (ACT), Prothrombin Time (PT), Thrombin Clotting Time (TT)] as well as more experimental assays [Ecarin Clotting Time (ECT), Prothrombinase-induced Clotting Time (PiCT)] are described and discussed. In considering the effects of melagatran on the coagulation assays, it is important to remember that the values reported for each test will depend on the reagents, methodology, and instrumentation used. Therefore, the objective of this paper is to consider the sensitivity and concentration—effect profile for the different coagulation tests with melagatran, and not to provide absolute values as targets for effective anticoagulation. The determination of melagatran concentrations in plasma is described before considering the effects on the various coagulation tests in vitro with melagatran and ex vivo following oral administration of ximelagatran to patients and volunteers.

Determination of plasma melagatran concentrations

The concentration of melagatran in plasma was determined by liquid chromatography (LC)-positive electrospray ionization mass spectrometry as previously described [22,23]. Melagatran is isolated from plasma by solid-phase extraction on octylsilica (absolute recovery>92%). Melagatran and an internal standard, melagatran $D_2^{13}C_2$, were separated from other sample components by LC utilizing a C_{18} stationary phase and a mobile phase comprising 35% acetonitrile and 0.08% formic acid in 0.0013 mol/l ammonium acetate solution. The relative standard deviation was 1—5% for concentrations above the limit of quantification (10 nmol/l).

Coagulation assays and the effects of ximelagatran and melagatran

Coagulation assays are the most commonly used method of monitoring anticoagulant therapy and screening for defects in the coagulation system. The endpoint for the assays is fibrin polymerization, which is determined on a coagulometer using an instrument-specific algorithm.

The effect of melagatran on the various coagulation assays has been investigated in vitro by the addition of defined concentrations of melagatran to plasma from healthy human volunteers. Furthermore, effects on the coagulation assays have been assessed ex vivo using plasma samples from healthy volunteers as well as from patients. Melagatran acts on thrombin-mediated conversion of fibrinogen to fibrin, and so effects on all coagulation assays would be expected. In vitro studies with melagatran and ex vivo studies with ximelagatran and melagatran