New oral anticoagulants have been shown to be not inferior to vitamin K antagonists in reducing thrombo-embolic events in patients with non-valvular atrial fibrillation and venous thrombo-embolism. However, among them which directly inhibit thrombin have been associated with greater risk of myocardial infarction. In this article we review the pleiotropic physiological effects of thrombin and their potential link with the observed greater incidence of myocardial infarction during therapy with oral direct thrombin inhibitors. On this basis, we believe that further studies are necessary to clear out doubts on the use of these drugs in the general population and, more specifically, in patients with coronary artery disease. For these reasons, in our opinion at present it may be prudent to especially caution high risk patients initiating therapy with a direct thrombin inhibitor (or those who are already taking it) about this possible risk. For patients with established coronary artery disease an alternative oral anticoagulant may be at present a better choice.

Keywords: new oral anticoagulants, oral direct thrombin inhibition, myocardial infarction, coronary artery disease, side effects.
al studies, mostly stemming from residual confounding despite propensity matching, may explain the discrepancy between the current observational studies and previous randomized trials (9).

Conversely, oral NOAcs acting at an earlier step along the coagulation cascade have not been related to increased risk of MI. Rivaroxaban, apixaban and edoxaban do not directly act on thrombin but inhibit activated factor Xa, leaving thrombin “untouched”. Thrombin, apart from converting soluble fibrinogen into insoluble strands of fibrin, catalyzes many other coagulation/anticoagulation-related reactions. In fact, the potential mechanisms beyond potential oral DTI-induced cardiac serious side effect has not been investigated yet. In the present article we review the potential actions of oral DTIs on potential pathophysiology of previously reported cardiac side effects.

**Potential pro-thrombotic effects of direct thrombin inhibition**

Thrombin, alternatively called activated factor II (IIa), is a short half-life serine protease, rapidly inactivated by heparin-antithrombin via “irreversible kinetic trap”. The best established role of thrombin is to favour primary and secondary haemostasis by cleaving platelet receptors PAR1 and PAR4, thereby promoting platelet activation, and by cleaving fibrinogen and activating FXIII and the consequent development of a stable platelet and fibrin plug (10). Thrombin also exerts a positive feedback on its generation and promotes inhibition of fibrinolysis by the release of plasminogen activator inhibitor 1 (PAI-1) and activation of thrombin-activatable fibrinolysis inhibitor (TAFI), which reduces the affinity of plasminogen for fibrin (11). Alongside pro-coagulation actions, thrombin presents crucial anti-coagulant and pro-fibrinolytic activities, which play an essential role in haemostasis control (12).

This could represent the “back side of the coin” of iatrogenic thrombin inhibition. There is a rationale for presuming that direct thrombin inhibition could not only prevent thrombosis in certain predisposing conditions - such as atrial fibrillation - but also favour thrombosis in other predisposing venues - such as arterial unstable plaques. For these reasons, we surmise that, due to its pleiotropic functions in the haemostasis cascade and its regulation, direct thrombin inhibition might be an unsafe target for pharmacological antagonism. Schematic thrombin activities are represented in Figure 1.

The most relevant thrombin-dependent anti-coagulant mechanism is protein C (PC) activation. PC is a circulating vitamin K-dependent zymogen and a substrate for proteolytic cleavage by thrombin. More precisely, thrombin binds with high affinity to thrombomodulin, a specific endothelial receptor, thereby assuming the quasi-selective role of targeting PC (13). Activated PC (APC), in conjunction with its cofactor protein S (PS), effectively inactivates by limited proteolysis the activated forms of factors VIII and V. Furthermore, APC directly inhibits PAI-1 and the APC-PS complex prevents TAFI activation (14, 15). Therefore, APC is a key regulator of clot formation and dissolution. Consequently, direct inhibition of thrombin might inhibit this crucial mechanism of thrombus prevention, thus possibly facilitating clot formation, especially on endothelial cells at risk, e.g. in correspondence of unstable plaques. In fact, low concentrations of the DTI melagatran and dabigatran have been shown to enhance thrombin generation and hypercoagulability, possibly via inhibition of the PC system (16). Figure 2 resumes hemostasis cascade with pharmacological targets.

Atherosclerotic plaques are subject to inflammation, which is thought to be a key point in determining the risk of thrombosis
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Figure 1 - Schematic representation of thrombin pleiotropic activities. FP = fibrinopeptides; PAI = plasminogen-activator inhibitor; PAR = protease-activated receptor; PC = protein C; TAFI = thrombin-activatable fibrinolysis inhibitor; t-PA = tissue-plasminogen activator; VWF = von Willebrand factor.

Figure 2 - Hemostasis cascade. Straight lines mean activation, dashed lines mean inactivation. Circles outline anticoagulants principal targets. Xa: unfractioned heparin, low-molecular-weight heparin, fondaparinux, warfarin and vitamin K antagonists, rivaroxaban, apixaban, edoxaban. Thrombin: unfractioned heparin, low-molecular-weight heparin (marginally), warfarin and vitamin K antagonists, dabigatran, ximelagatran, AZD0837.

HMWK = high-molecular-weight kininogen; TFPI = tissue factor pathway inhibitor.
on the plaque itself (17). Endothelial cells, smooth muscle cells and lipidic core macrophages express high levels of tissue factor (TF) (18), the major trigger of secondary haemostasis. In addition to its anti-coagulant activity, APC presents a variegated anti-inflammatory activity that further increases its capability to prevent thrombus formation (19). For example, it interferes with leukocyte adhesion to E-selectin, reduces monocyte-dependent cytokine release and inhibits leukocyte TF production. Thrombin inhibition might therefore interfere also with these important atherosclerosis counter-regulatory mechanisms.

Thrombin stimulates the release of t-PA, the most relevant plasminogen activator (20). t-PA converts plasminogen into plasmin, a non-selective enzyme that cuts fibrin after its deposition in the thrombus. Thus, the pro-fibrinolytic activity of thrombin may be affected by oral DTIs. Thrombin inhibitors may also facilitate platelet adhesion and activation during primary haemostasis, given that thrombin favours endothelial cells to release prostacyclin (21), a powerful platelet adhesion inhibitor.

As a consequence of fibrotic cap rupture, hemostasis cascade is activated on unstable atherosclerotic plaques. Its progression towards occlusive thrombus or not is a matter of TF concentration and predominance of pro-coagulant versus anti-coagulant factors. A key point is the dynamic equilibrium between PAI-1 and t-PA concentrations, in which thrombin plays a master role. Direct pharmacological inhibition of thrombin inevitably interferes with these mechanisms.

**Comparison of indirect versus direct thrombin inhibition**

Oral DTIs, as well as heparin, and differently from vitamin K antagonists, are able to inactivate both fluid-phase and membrane-bound thrombin, which reflects the ability to dissolve organized thrombi. Thrombin is inactivated by heparin-anti-thrombin via “irreversible kinetic trap”, which is a physiological thrombin counter-regulatory mechanism but also a pharmacological anticoagulant strategy realized through heparin polysaccharides parenteral administration. Heparin is a well known, variable molecular weight, negative-charge enriched glycosaminoglycan which binds to antithrombin and allows it to indirectly inactivate thrombin (by “trapping” it on phospholypidic membranes) and directly inactivate factor Xa and other hemostasis factors such as XIIa, XIa, IXa and VIIa (22). Since thrombin and Xa are produced in higher concentrations than other factors, heparin’s anticoagulant indirect (e.g. through antithrombin) activity is mostly elicited through thrombin and Xa inhibition.

In the context of atrial fibrillation, vitamin K antagonists have always been preferred to heparin due to oral administration which allows long-term treatment. Heparin’s anticoagulation, differently from vitamin K antagonists effects, is virtually immediate since it is not affected by hemostasis factors half-life but only by its own kynetics. Heparin use is generally limited to the initial phase treatment or prophylaxis, followed by bridging to oral vitamin K antagonists. Heparins’ anti-Xa/anti-IIa ratios, as well as their half-lives, are inversely proportional to their molecular weights (23). Pharmacologically available forms of heparin have widely different molecular weights, ranging from unfractioned heparin (UFH) to many different low-molecular-weight heparins (LMWHs) and to fondaparinux, which represents the minimal essential antithrombin-activating pentasaccharidic chain. UFH acts both on thrombin and Xa, whereas LMWHs are quasi-selective towards Xa and fondaparinux is absolutely selective towards Xa. Whether UFH is associated with a greater risk of MI compared
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Metabolic effects of thrombin inhibition

Despite beneficial anticoagulant effects, UFH administration is also known to increase circulating free fatty acids (FFA) (26), which may adversely affect myocardial energetics, especially during ischemia (27). In the past, standard unfractionated heparin has been shown to reduce the ischemic threshold in patients with coronary artery disease (CAD), probably by increasing FFA release (28). Pharmacological partial inhibition of fatty acids oxidation has been shown to reduce this deleterious effect of heparin (29).

Besides altering the metabolic balance of ischemic myocardium, increased FFA release may also adversely affect the endothelial function (30). It has been shown that under physiological conditions coronary endothelial cells predominantly utilize exogenous glucose for energy production (31). In these cells glucose effectively suppresses the oxidation of lactate and palmitate, which are the preferred substrates for the heart muscle. Similarly to the cardiac muscle, metabolism of endothelial cells may be negatively affected by ischemia and increased availability of FFA.

Similar to unfractionated heparin, direct thrombin inhibition has been shown to enhance peripheral lipolysis (32). If this were the case, beside the above mentioned mechanisms, high levels of circulating FFA could decrease cardiac metabolic efficiency in the setting of acute coronary syndrome, in patients on therapy with oral DTI. However, this hypothesis needs confirmation.

Potential maladaptive vascular remodeling by direct thrombin inhibition

It has been recently shown that thrombin and plasmin can cleave chromogranin A, an anti-angiogenic cardio-regulatory protein released in the blood by the neuroendocrine system (and by the heart itself) (33, 34). Chromogranin A can also work as a precursor of peptides with opposite functions on vascular and heart contractility, such as vasostatin-1, catestatin, and serpinin (34, 35). Notably, cleavage of CgA by thrombin converts chromogranin A into a fragment capable of inducing the release of basic fibroblast growth factor (bFGF) and, therefore, of promoting angiogenesis (34). On the other hand vasostatin-1, another fragment of CgA, which can be generated by plasmin, is a potent anti-angiogenic factor and a negative regulator of the endothelial barrier function (34). Both precursor and fragments are present in the blood to form a balance of anti-/pro-angiogenic molecules tightly regulated by proteolysis. Oral DTIs may interfere in an unpredictable manner in this balance, with potentially important consequences for the regulation of vascular remodeling in atherosclerotic coronary arteries.

CONCLUSION

Most studies employing oral DTIs have shown an association with myocardial infarction occurrence. However, the available information is not yet sufficient to express a definitive judgement on the use of oral
DTIs in patients with or at risk of coronary artery disease. Considering the pleiotropic physiological effects of thrombin discussed above and the reported greater incidence of myocardial infarction during therapy with oral DTIs, we believe that further studies are definitely necessary to clear out the doubts on the use of these drugs in the general population and, more specifically, in patients with coronary artery disease. For these reasons, in our opinion it may be prudent at present to especially caution high risk patients being started on a direct thrombin inhibitor (or those who are already taking it) about this possible risk. For patients with already established coronary artery disease an alternative oral anticoagulant may be at present a better choice.

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