Differential cellular effects of old and new oral anticoagulants: consequences to the genesis and progression of atherosclerosis

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Summary
The main purpose of anticoagulants is to diminish fibrin formation, thereby decreasing the risk of venous or arterial thrombosis. Vitamin K antagonist have been used for many decades in order to achieve reduced thrombotic risk, despite major drawbacks of this class of drugs such as cumbersome dosing and monitoring of anticoagulant status. To overcome these drawbacks of VKA, new classes of anticoagulants have been developed including oral anticoagulants for direct inhibition of either thrombin or factor Xa, which can be administered in a fixed dose without monitoring. Coagulation factors can activate cellular protease-activated receptors, thereby inducing cellular processes as inflammation, apoptosis, migration, and fibrosis. Therefore, inhibition of coagulation proteases not only attenuates fibrin formation, but may also influence pathophysiological processes like vascular calcification and atherosclerosis. Animal models revealed that VKA therapy induced both intima and media calcification and accelerated plaque vulnerability, whereas specific and direct inhibition of thrombin or factor Xa attenuated atherosclerosis. In this review we provide an overview of old and new oral anticoagulants, as well discuss potential pleiotropic effects with regard to calcification and atherosclerosis. Although translation from animal model to clinical patients seems difficult at first sight, effort should be made to fully understand the clinical implications of long-term oral anticoagulant therapy on vascular side effects.

Keywords
Arterial thrombosis, arteriogenesis, atherothrombosis, vitamin K-dependent factors, atherosclerosis

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Oral anticoagulants

Vitamin K-antagonists (VKA) and heparins have been anticoagulants for prevention and treatment of thromboembolic events for some 70 years. VKA are still the most widely used anticoagulant drugs to treat patients at risk of arterial and venous thrombosis. Due to unfavorable pharmacokinetics of VKA, direct thrombin and factor Xa (FXa) inhibitors (non-vitamin K-antagonist oral anticoagulants, NOACs) have been introduced into the clinic as alternatives to VKA. Today, it is still a matter of debate which treatment is superior with respect to bleeding risk. Anticoagulant drugs also influence non-haemostatic activities of coagulation factors. Experimental data indicate that thrombin can induce an array of pro-atherogenic and plaque-destabilizing effects. Together with the clinical safety of long-term treatment with VKA this suggests that they have also protective effects on the vasculature, possibly via protease-activated receptors (PARs) and via vascular vitamin K-dependent proteins. Knowledge of these as yet unknown effects of VKA and of NOAC will help to improve the long-term prophylaxis and treatment of arterial and venous thrombosis, and will support personalised medicine.

History
The story of oral anticoagulants starts in the early 1920s. Oral anticoagulants were discovered in the malady of cattle involving fatal bleeding (1). Cattle being dehorned that had eaten spoiled hay had little to no clotting power resulting in fatal haemorrhages after 30–50 days. It was Roderick who showed in 1931 that the abolished coagulability of the blood was due to a prothrombin deficit.

In the late 1930s Campbell (2) isolated and purified crystalline dicoumarol from the hay. After synthetic dicoumarol became available in quantity, the essentials of its physiologic action were quickly established. Due to the lag period of dicoumarol, probably due to variability in absorption kinetics, it was anticipated that the action would largely vary within human individuals (3). Dicoumarol was used in trials for its rodenticidal purpose. However, the activity of dicoumarol was not high enough, which was due to poor and variable absorption kinetics, weak antagonist of vitamin K, and the vitamin K content of grains and green foods. In the following years, over 100 related dicoumarol compounds were prepared, with number 42 being the one with the highest anticoagulant activity, and this was promoted for rodent control under the auspices of Wisconsin Alumni Research Foundation. Hence, the name...
Vitamin K was discovered in the 1930s by Henrik Dam. It was serendipity that Dam found this “antihaemorraghic factor” as he was investigating cholesterol metabolism in chickens which developed unexpectedly large subcutaneous and intramuscular haemorrhages (4). Dam coined this micronutrient Koagulation factor (abbreviated as vitamin K), because of its requirement for normal haemostasis. It took until 1974 before the unequivocal role of vitamin K action was established as cofactor for the posttranslational carboxylation of glutamate residues (5). The substrates of gamma-glutamyl carboxylase are the so-called vitamin K–dependent proteins – coagulation factors prothrombin (II), X, VII, IX, protein C, and protein S, which are involved in various physiological processes such as blood coagulation (Figure 1), bone and soft tissue mineralisation, and cellular proliferation. The coagulation factors II (prothrombin), VII, IX, and X all have procoagulant activity, whereas proteins C and S inhibit blood coagulation (reviewed in [6]). Four key components are involved during the post-translational modification of the vitamin K–dependent proteins: the enzymes gamma-glutamyl carboxylase and vitamin K epoxide reductase complex (VKOR), vitamin K, and a precursor protein (Figure 2). The discovery of the unusual amino acid γ-carboxyglutamic acid (Gla) (7) in prothrombin (8) as the product of vitamin K-action was the first hint on the action of VKA. Matschiner et al. showed that administration of warfarin resulted in a significant increase of the epoxide form of vitamin K in rats (9). In brief, vitamin K derived from food products (vitamin K quinone) has little to no cofactor activity and needs to be converted into the active cofactor vitamin K hydroquinone (KH₂₂, Figure 2). During carboxylation, vitamin KH₂ is converted into vitamin K epoxide (KO), which subsequently will be reduced to vitamin K and vitamin KH₂ in two reactions by the action of the enzyme vitamin K-epoxide reductase (VKOR). The VKOR gene was identified in 2004, when both the lab of Stafford (10) and Oldenburg (11) published the purification of the gene coding for the enzyme VKOR. This recycling mechanism is called the vitamin K cycle and the VKA specifically inhibit the enzymatic activity of VKOR, thereby causing a decrease in the active cofactor vitamin KH₂₂, resulting in attenuated gamma-glutamyl carboxylation. Through the action of gamma-glutamyl carboxylase, the vitamin K-dependent coagulation factors (procoagulants: prothrombin, factors VII, IX, and X, and anticoagulants: protein C and S) gain a calcium-specific membrane binding domain (also known as Gla-domain), enabling the high affinity binding to an anionic-rich phospholipid bilayer. Assembling the key players in coagulation on an anionic phospholipid bilayer increases the local concentrations of both enzyme and substrate, thereby decreasing the apparent Km far below their plasma concentration. However, this drop in Km is not translated into an increase in zymogen conversion and the cofactors Va and VIIIa are needed in order to enhance the enzymatic activity (Vmax). In summary, organisation of the coagulation factors on an anionic phospholipid bilayer and the activities of cofactors V and VIII, causes a dramatic acceleration of the velocity at which factor X and prothrombin are activated by the tenase (factors IXa, VIIIa, and X) and prothrombinase

**Figure 1: Effects of vitamin K antagonists (VKA) and direct oral anticoagulants (NOAC) on coagulation, e.g. fibrin formation, and vascular proteins.** Whereas VKA interfere with synthesis of all vitamin K–dependent proteins – coagulation factors prothrombin (II), X, VII, IX, protein C, and protein S, matrix Gla protein (MGP, and Gas6), NOACs were developed for the specific inhibition of either thrombin (IIa) or factor Xa.
complex (factors Xa, Va, and prothrombin), respectively. Therefore, inhibition of gamma-glutamyl carboxylation leading to disrupted membrane-binding capacity for coagulation factors results in diminished haemostasis.

**Coumarins**

Since the mid 1950s, VKA has been given to patients worldwide and still form the treatment basis of contemporary thromboprophylaxis (12). Today, several VKAs are used in the clinical setting all belonging to the class of compounds called 4-hydroxycoumarins or 1,3 indandiones (13). The major 4-hydro-xycoumarin anticoagulants in current use are warfarin, acenocoumarol, and phenprocoumon. All VKAs act indirectly on inhibition of carboxylation of vitamin K-dependent proteins. The most important complication of treatment with VKA is haemorrhage, which may be life-threatening (14) as it increases the risk of major bleeding by 0.5%/year (15).

Despite the effectiveness of VKA, treatment has several shortcomings. First, the activity of VKA on anticoagulation potential has to be monitored by blood testing. Secondly, many commonly used medications interact with VKA. Thirdly, large amounts of vitamin K in particular in green leafy vegetables are well absorbed in the intestinal tract, thereby counteracting VKAs in the liver (16). Finally, VKA are not restricted to vitamin K-dependent coagulation factors and inhibit vitamin K-dependent proteins involved in other physiological processes, such as bone metabolism and vascular function (17), as well. The best studied side-effect of VKA is on matrix Gla-protein (MGP), a strong inhibitor of vascular calcification (reviewed in [18]).

Due to these unfavourable pharmacokinetics and actions of VKA, direct thrombin and factor Xa (FXa) inhibitors (NOACs) have been introduced as alternatives to VKA.

**Non vitamin K antagonist oral anticoagulants**

Non vitamin K antagonist oral anticoagulants (NOACs) are small synthetically made molecules against one clotting factor (i.e. thrombin or factor IIa) or factor Xa. They bind selectively and with high affinity to the active site of the enzyme, thereby inhibiting the turnover of the natural substrates. Direct thrombin inhibition is straightforward by inhibition of the key regulator of coagulation. Factor Xa inhibition is based on reducing the thrombin burst in the propagation of coagulation.

**Thrombin inhibitors**

The story of thrombin inhibitors goes back to the late 1950s when hirudin, produced by the medicinal leech Hirudo medicinalis, was isolated and biochemically characterised as a potent thrombin inhibitor. Hirudin forms a 1:1 stoichiometric complex with thrombin through binding to exosite 1 (anion-binding or fibrinogen binding site) and the active catalytic domain of the protease (19). With a dissociation equilibrium constant (K<sub>i</sub>) of 20 FM, hirudin is still the most potent thrombin inhibitor. Using recombinant DNA technology structural variants of the native hirudin were produced (reviewed in [20]). Although hirudin analogs are approved for clinical use, their use is limited because they are not orally active. Ximelagatran, a pro-drug of melagatran, was the first orally active thrombin inhibitor. Melagatran directly targets the active site of thrombin. Ximelagatran has been evaluated extensively for the replacement of warfarin in atrial fibrillation (AF) patients. Despite favorable results, ximelagatran did not make it for clinical use due to hepatotoxicity. Dabigatran etexilate, a prodrug of dabigatran, was the second orally thrombin inhibitor. It is a potent, competitive, reversible direct thrombin inhibitor directed against the active site of thrombin, and capable of inactivating both fibrin-bound and unbound thrombin.

**Direct factor Xa inhibitors**

Factor Xa inhibitors were discovered in the early 1980s. The first factor Xa inhibitor, called Antistasin, was isolated from the salivary glands of the Mexican leech Haementeria officinalis. Antistasin is a polypeptide with a slow and tight-binding, and thus potent factor Xa inhibitor (21). A second naturally occurring factor Xa inhibitor, isolated from extracts of the soft tick Ornithodoros moubata, is the tick anticoagulant peptide (TAP), a single-chain, 60 amino-acid peptide. Inhibition of factor Xa by recombinant TAP showed sustained in vitro and in vivo inhibition of clot-associated procoagulant activity. These data provided the support for the concept of factor Xa inhibition. Subsequently, chemical companies inspired by this coagulation inhibition of factor Xa, initiated chemistry programs to develop small synthetic factor Xa inhibitors, such as rivaroxaban, apixaban, betrixaban, and edoxaban. Rivaroxaban is the first most advanced synthetic factor Xa inhibitor with a 10,000-fold higher selectivity for factor Xa than other serine proteases. Inhibition of factor Xa is concentration dependent and affects prothrombinase complex bound as well as clot-associated factor Xa.

**Stroke prevention in AF patients: VKA vs NOACs**

Atrial fibrillation (AF) is the predominant indication for long-term anticoagulant treatment. According to AF-guidelines, indication for long anticoagulant treatment is based on the stroke risk, which can be estimated using the CHADS<sub>2</sub> score (22). CHADS<sub>2</sub> stands for congestive heart failure, hypertension, age ≥ 75 years and stroke/transient ischaemic attack. Each risk factor of the CHADS<sub>2</sub> score accounts for one point except for stroke/transient ischaemic attack which accounts for two points. The higher the CHADS<sub>2</sub> score the higher the risk of thrombo-embolic complications and as a result long-term anticoagulant treatment is indicated in patients with a CHADS<sub>2</sub> score of ≥1 according to European and Canadian guidelines and in patients with a CHADS<sub>2</sub> score of ≥2 according to American guidelines (23). Several major trials designed to test whether direct thrombin or factor Xa inhibitors were non-inferior to VKAs with respect to efficacy are...
In the RELY trial, 18,113 subjects with AF were enrolled and randomly assigned to receive fixed dose of dabigatran (direct factor IIa inhibitor), 110 mg or 150 mg twice daily, or dose adjusted VKA (26). Administration of 110 mg dabigatran showed similar results as compared to VKA with respect to stroke and systemic embolism; however, with lower rates of major haemorrhage. The 150 mg dose of dabigatran was associated with lower rates of stroke and systemic embolism, but comparable rates of major haemorrhage. In the ROCKET-AF study, 14,264 randomly assigned patients with AF who were at increased risk for stroke received rivaroxaban (direct Xa inhibitor) or dose adjusted VKA (27). Rivaroxaban showed to be non-inferior to VKA for the prevention of stroke or systemic embolism. And although no significant difference in risk for major bleeding was found, less intracranial and fatal bleedings occurred in the rivaroxaban group. These results were reinforced by the ARISTOTLE trial in which 18,201 subjects with AF and at least one risk factor for stroke were enrolled and randomly assigned to receive apixaban 5 mg twice daily (factor Xa inhibitor) or VKA (24). Instead of finding non-inferiority of apixaban to VKA, the investigators found that apixaban was superior to VKA with respect to reduction of stroke risk or systemic thromboembolism by 21% and risk of bleeding by 31%. Furthermore, all cause death, as compared to VKA, was reduced by 11%. Thus far, dabigatran etexilate, rivaroxaban and apixaban all have shown to be non-inferior to VKAs with apixaban being superior to VKA.

**Figure 2: Anticoagulants have different effects on activation of protease activated receptors (PARs).** A) The vitamin K cycle. Vitamin K-quinone (K) is first reduced to vitamin K-hydroquinone (KH2), and then oxidised to vitamin K-epoxide (KO). The vitamin K-epoxide reductase (VKOR) enzyme converts the epoxide form back to the quinone form, thereby completing the vitamin K cycle. During the oxidation step glutamic acid (Glu) is reduced to gamma-carboxy glutamic acid (Gla) by the enzyme gamma-glutamyl carboxylase. The VKOR enzyme is inhibited by vitamin K antagonists (VKA), causing diminished carboxylation. As a result, the liver secretes the vitamin K-dependent proteins in their precursor form, without the correct membrane binding domain. Activated coagulation factor X without the membrane binding domain retained the capability to activate PARs. B) Direct factor Xa inhibitors attenuate the activity of the protease, which as a consequence can not activate prothrombin and the cellular PARs. C) Direct thrombin inhibitors attenuate the activity of the protease, which as a consequence can not activate cellular PARs, whereas factor Xa remains unaffected and can activate the PARs.
Major advantages of all NOACs over VKA are the stable pharmacokinetics and –dynamics, which allows fixed dosing in all patients. Consequently frequent laboratory monitoring is redundant. However, there are a number of clinical situations in which laboratory monitoring might be advisable: such as in non-compliance, therapy failure and major bleeding. Moreover, in patients with extremely low or high body mass index, in poly-pharmacia or in the presence of comorbidity the possibility to check the activity of a prescribed drug might be advantageous. However, at the moment there are no laboratory tests available to monitor the direct anticoagulants in practice. In addition, unlike for VKA no antidotes exist to reverse the effects of direct thrombin or Xa inhibitors.

Coagulation and atherosclerosis

Although the pharmacological effects of anticoagulants are clear and have been described in detail elsewhere, long-term treatment with VKA or the NOACs, such as dabigatran and rivaroxaban, may have unwanted side effects. In a recent review published on the haemostatic system as a modulator of atherosclerosis, thrombin and factor Xa are believed to be potential modulators of plaque phenotype, as they are involved in inflammation, endothelial function, vasoconstrictor tone and many more (28). Both factors signal through a family of cellular PARs (PAR1 to –4). PARs are activated by proteolytic cleavage and all PARs, except PAR2, are activated directly or signal directly in response to thrombin. However, thrombin-cleaved PAR1 can donate its tethered ligand to transactivate PAR2, which in turn has been shown to modulate the PAR1-induced hyperplastic response to arterial damage preceding stenosis (29) and PAR1-mediated tumour cell migration and metastasis (30). However, direct thrombin-induced signalling responses through PAR1 activation seem to be different from the transactivation of PAR1 and PAR2 heterodimers (31). Also, formation of PAR1 and PAR4 heterodimers dependent on thrombin-induced cleavage of both receptors has also been reported (32). Factor Xa-dependent cellular responses can occur through PAR1 and/or PAR2 cleavage, and these processes may depend on the receptor-specific cell expression pattern, ligand concentration, solubility, or association with other coagulation factors (33). Overall, the proteolytic activation of PARs by either thrombin or factor Xa results in the activation of a canonical G-protein pathway and, consequently, of downstream signalling pathways that influence multiple transcription-regulated, cell-specific events including proliferation, inflammation, migration, adhesion, and apoptosis (28). Through this modulation of cell behavior, activation of PARs by thrombin or factor Xa may contribute to the development and progression of pathologic conditions including arthritis, fibrotic lung disease, cancer, and atherosclerosis (33–35). In relation to atherosclerosis, diminished coagulation has been shown to attenuate atherosclerosis (reviewed in [36]), and these observations are supported by experiments demonstrating that the direct cellular effects of factor Xa and thrombin can be responsible for promoting inflammation, leukocyte transendothelial migration, angiogenesis, narrowing of blood vessels, as well as migration and proliferation of VSMCs (28). PAR4-mediated activation of platelets by thrombin has been suggested to have no contribution to development of early atherosclerotic lesions (37). Not only does PAR4 require high concentrations of thrombin or PAR3 as a cofactor in activation (38), a study with PAR4 deficient mice on an atherosclerotic background showed no significant differences in the percentage of aortic luminal surface covered by plaques and in the cross-sectional area of plaques compared to ApoE<sup>–/–</sup> mice after five and 10 weeks on a Western diet (37).

Supporting the theory of a relation between coagulation and development and progression of atherosclerosis is the presence of coagulation factors and corresponding proteolytical activity in atherosclerotic lesions (39, 40), with increased activities in early atherosclerotic lesions compared with lesions at a later stage (41). Indeed, increased (hypercoagulability) and diminished (hypocoagulability) coagulation have been suggested to contribute to development of atherosclerosis, as suggested from several animal studies. A study from our group showed enhanced atherosclerosis with a higher plaque vulnerability, spontaneous atherothrombosis, enhanced oxidative stress, neutrophil intraplaque infiltration and apoptosis, and diminished VSMC proliferation in animals on a combined atherogenic and hypercoagulable background (42). A hypocoagulable phenotype on an atherogenic background showed reduced atherosclerosis, decreased leukocyte infiltration and altered collagen and VSMC content (42). These results confirmed earlier observations showing that hypocoagulable mice double deficient in factor VIII on an atherogenic background showed significantly less early-stage atherosclerotic lesions (43) and that hypercoagulable mice (either deficient in the natural anticoagulant heparin cofactor II or tissue factor pathway inhibitor) showed a larger total atherosclerotic plaque area or atherosclerotic burden while on an atherogenic background (44, 45). Remarkably, a 50% reduction of tissue factor expression in ApoE<sup>–/–</sup> mice did not result in a change of the atherosclerosis extent compared to wild-type mice (46). Besides animal studies, several clinical studies suggest both a hypercoagulable state and active thrombin generation in association with cardiovascular disease and atherosclerosis (reviewed in [47]). However, a direct relationship between a hypercoagulable state and development and progression of atherosclerosis is not immediately evident from clinical studies. The association between hypercoagulability-related common thrombophilic gene defects, such as prothrombin G20210A mutation, FV Leiden mutation, and deficiencies in protein C, protein S, or antithrombin (AT), and cardiovascular disease are modest. Besides, reported associations are mainly observed in young patients (age < 45 years), at onset of disease before the age of 55 years (and in the absence of usual risk factors), or with early occlusion after revascularisation procedures (48, 49). Comparable conclusions can be drawn for hypocoagulability and association with cardiovascular. Although, haemophilic patients have a two- to three-fold increased overall mortality from bleeding disorders, liver diseases, or viral infections, the effects of factor deficiency on cardiovascular mortality resulting from ischaemic heart remain controversial. One possible confounder in the different observations might be the therapeutic administration of coagulation proteins in patients...
with severe haemophilia, potentially obscuring the effects of the original factor deficiency. Hypercoagulability can also be defined as having elevated circulating markers of activated coagulation such as prothrombin fragment 1.2, thrombin:antithrombin complexes, or D-dimer. Overall, plasma levels of markers of active coagulation are elevated in relation to the extent of atherosclerosis, and have been shown to be predictive of cardiovascular events in some studies (50). More precisely, several clinical studies have demonstrated that the specific fibrin degradation product D-dimer is associated with an increased risk of severe atherosclerosis and vascular complications (51–59). Prothrombin fragment 1.2 has been shown to be independently associated with carotid intima media thickness (IMT) in adults without clinically overt atherosclerotic disease (60). Furthermore, the presence of coronary atherosclerosis was associated with increased levels of the prothrombin activation markers prothrombin fragment 1.2 and thrombin:antithrombin complex (61) and the size of atherosclerotic plaques positively correlated with prothrombin fragment 1.2 in patients with a previous myocardial infarction (62) and first ischaemic stroke (63). Data from our own group showed that elevated thrombin:antithrombin levels were independently associated with the presence and severity of coronary atherosclerotic plaques, but also with the degree of vascular calcification, as defined by coronary computed tomographic angiography (64).

In conclusion, a clear contribution of coagulation proteases to the development and progression of atherosclerosis has been demonstrated, whereas in humans with atherosclerosis increased coagulation activity is present in circulation and within atherosclerotic lesions. It remains, however, to be elucidated if hypercoagulability in humans is causally related to structural changes in the arterial vessel wall contributing to atherosclerosis. Nevertheless, given the strong evidence obtained from both experimental studies and animal models, it seems reasonable to hypothesise that long-term treatment with anticoagulants might have beneficial or harmful outcomes.

**Pleiotropic effects of anticoagulants**

**Calcification**

As mentioned above long term treatment with VKA induces and accelerates vascular calcification. The first pre-clinical data are from the mid-1990s, in which the combined treatment of vitamin K with VKA resulted in a severe vascular vitamin K deficiency, with concomitant medial vascular calcification (65). Recently it was shown that in apoE/ mice bearing atherosclerotic plaques VKA also induced accelerated intimal calcification and increased features of plaque vulnerability (66). Additionally, mice on VKA treatment showed augmented aortic peak velocity, aortic valve–peak gradient, and carotid pulse-wave velocity (67).

It took until 2004 before the effect of VKA on vascular calcification in the clinical setting was revealed (68). Today, the association between VKA use and vascular calcification has been confirmed in several trials (17). As mentioned above, AF is the predominant indication for long-term anticoagulant treatment. In patients with atrial fibrillation coronary artery calcification was investigated in relation to treatment and duration of VKA treatment (69). Low-risk AF patients (those with a CHADS2 score below 1) using a VKA had more calcification and atherosclerosis than those patients not treated with VKA. Moreover, duration of treatment was also significantly correlated with increased coronary artery calcium score. However, due to the high efficacy of VKA in stroke prevention, the potential harmful effects of VKA-associated vascular calcification might be offset by the beneficial effect, especially in patients with high stroke risk. For that reason the clinical importance of VKA-induced vascular calcifications remains unclear, and needs to be more personalised.

Unlike VKA the direct anticoagulant drugs possibly do not interfere with functionality of MGP (18) and prospective observational studies including AF patients treated with either VKAs or new anticoagulant drugs might reveal the importance of VKA-associated vascular calcifications in the long term (17). Some degree of vascular inflammation is concomitant with most forms of calcification. Monocytes and macrophages have been observed to enhance vascular calcification via both cell–cell interaction and the production of soluble factors. Inflammatory cytokines such as interleukin (IL)-1β, IL-6, IL-8, insulin-like growth factor (IGF)-1 and tumour necrosis factor (TNF) induce the osteogenic switch of vascular smooth muscle cells (VSMCs), thereby enhancing calcification (70–72). Nadra et al. (71) showed that basic calcium phosphate crystals are taken up by macrophages, and that this was associated with release of TNF, IL-1β and IL-8. Additionally, calcium phosphate induced macrophage IL-1β secretion through activation of the NLRP3 inflammasome (73). Also VSMCs are professional phagocytic cells, and thus can take up calcium crystals. This results in apoptosis for VSMCs thereby increasing the inflammatory response in the vasculature (74). Acceleration of calcification by VKAs administration may exert anti-inflammatory effects as well.

Although the direct relationship between vascular calcification and coagulation is still open for investigation, we can only speculate on how coagulation and calcification influence one another. One possible relationship can go via microvesicles (MVs) secreted by VSMCs. MVs, which are increasingly secreted upon calcification, can enhance calcification via both cell–cell interaction and the production of soluble factors. Inflammatory cytokines such as interleukin-1β, interleukin-6, interleukin-8, insulin-like growth factor (IGF)-1, and tumour necrosis factor (TNF) induce the osteogenic switch of vascular smooth muscle cells (VSMCs), thereby enhancing calcification (70–72). Nadra et al. (71) showed that basic calcium phosphate crystals are taken up by macrophages, and that this was associated with release of TNF, IL-1β and IL-8. Additionally, calcium phosphate induced macrophage IL-1β secretion through activation of the NLRP3 inflammasome (73). Also VSMCs are professional phagocytic cells, and thus can take up calcium crystals. This results in apoptosis for VSMCs thereby increasing the inflammatory response in the vasculature (74). Acceleration of calcification by VKAs administration may exert anti-inflammatory effects as well.

**Atherosclerosis**

Pleiotropic effects of NOACs on atherosclerosis or atherothrombosis have been suggested from both animal studies and clinical trials. An increased frequency of myocardial infarctions has been reported in some (but not all) trials using direct thrombin inhibitors (26, 77). Moreover, in patients treated with ximelagatran increased markers of inflammation, such as IL-10 and C-reactive protein (CRP), were observed (78). Nonetheless in the latest AF guidelines, dabigatran is recommended instead of VKA or as an alternative for VKA for the prevention of stroke. Animal models...
have shown beneficial effects for NOACs on the development and progression of atherosclerosis. One of the first studies exploring the effects of direct thrombin inhibition on atherosclerosis was performed in ApoE-/- mice applying ximelagatran. The direct inhibition of thrombin caused enhanced stability of advanced atherosclerotic lesions characterised by thicker fibrous caps, smaller necrotic cores, and a decrease in matrix-metalloproteinase-9 (MMP-9) (79). Treatment of ApoE-/- mice with dabigatran resulted in comparable results with impaired formation and reduced size of atherosclerotic lesions (80). On a combined hypercoagulable and atherogenic background dabigatran reduced the pro-inflammatory and pro-atherogenic phenotype of atherosclerotic lesions, resulting in enhanced plaque stability (42). In this later model, leukocyte recruitment was attenuated and severe progression of atherosclerosis and atherothrombosis was prevented through direct thrombin inhibition. Comparable animal studies with direct factor Xa inhibitors showed comparable effects on atherosclerosis. For instance, selective inhibition of factor Xa through recombinant antistasin or tick anticoagulant peptide in rabbits was shown to reduced restenosis after balloon angioplasty of atherosclerotic femoral arteries and limit the narrowing of these vessels by atherosclerotic lesions (81). Direct factor Xa inhibition through administration of rivaroxaban has been shown to increase the stability of advanced atherosclerotic plaques in apolipoprotein E-deficient mice, as suggested by the presence of thicker protective fibrous caps and decreased erosion of the plaques (35). In addition, factor Xa inhibition reduce the expression of inflammatory mediators, such as IL-6, TNF-α and monocyte chemoattractant protein (MCP)-1, pointing towards atherosclerotic plaque stabilisation. However, rivaroxaban plasma levels in this model were rather low and the effects of clinical plasma concentrations needs to be established.

Conclusions

Although both VKA and NOACs act as anticoagulants, their mode of action suggests differences in pleiotropic effects. Whereas VKA treatment is mainly associated with enhanced calcification, NOACs treatment might have an effect on atherosclerosis progression in humans via PAR signalling. Coagulation factors produced in the presence of VKA are synthesised without the Gla domain, which is needed for binding to negatively charged cell membranes. These (so called) proteins induced by vitamin K antagonism or absence (PIVKAs) (82) cannot bind to negatively charged cell membranes. As a consequence, PIVKA-factor Xa will only convert minimal amounts of prothrombin into thrombin. However, the activation of PARs by coagulation factors has been suggested to occur in a membrane-binding independent way (83), suggesting that PIVKA factor Xa can still activate PARs (Figure 2). Treatment of cultured fibroblasts with full-length factor Xa or a truncated form of factor Xa lacking the membrane-binding domain (e.g. PIVKA factor Xa) revealed that both forms of the protease activated PARs (84), suggesting that factor Xa synthesis in the presence of VKA can still induce cellular responses (Figure 2).

In contrast inhibition of factor Xa using a direct inhibitor will inhibit not only the conversion of prothrombin to thrombin but also the factor Xa mediated activation of PARs. In the case of a direct thrombin inhibitor, fibrin formation and other functions of thrombin will be diminished, whereas factor Xa can still be generated by the tissue factor:factor VIIa complex (Figure 2). These differences in anticoagulant mechanisms provide in part an explanation for the observed differences in pleiotropic effects.

With an increasing demand for risk assessment of the individual patient and upcoming of new anticoagulants, the interplay between coagulation, inflammation and calcification deserves more studies to address its implications in risk. Although inhibiting factor Xa and thrombin may provide additional therapeutic potential over the VKA, data is still limited and mainly derived from experimental animal studies. The translation from animal to humans needs to be made, especially for the long-term effects of anticoagulant therapy. Therefore, further investigation is needed to fully understand the complexity and possible long-term benefits or drawbacks of both direct and indirect inhibition of coagulation proteases.

Conflicts of interest

None declared.

References


