Research Article

Pleiotropic Antithrombotic Effects of Cardiovascular Drugs

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Abstract

Cardiovascular drugs are cornerstones of treatment of various cardiovascular diseases in clinical and outpatient practice. Commonly used cardiovascular drugs such as statins and antihypertensive medication rank among the top 10 prescribed drugs in the United States. Although primarily administered to lower blood lipid, blood sugar or blood pressure levels, many of the drugs, administered to cardiovascular patients, have been reported to exert ancillary effects that contribute to their favorable risk-benefit profiles. The aim of this non-systematic review is to give an overview about the pleiotropic antithrombotic effects of cardiovascular drugs, and to discuss the potential underlying biochemical mechanisms and their clinical relevance.

Keywords: Pleiotropic; Antithrombotic effects; Cardiovascular drugs; Statins; Antidiabetic drugs

1. Introduction

Antithrombotic drugs such as aspirin, clopidogrel, prasugrel, ticagrelor among others are standardly used in patients with acute or chronic coronary syndrome, peripheral artery disease or cerebral stroke. Regarding prototypical antiplatelet drugs, there is plenty of knowledge and awareness about the underlying mode of action and the potential complications, predominantly major bleedings. Atherosclerotic cardiovascular disease (ASCVD) however, is a multifactorial disorder. Hyperlipidemia, arterial hypertension and diabetes represent important and treatable cardiovascular risk factors. In addition, ASCVD is often accompanied by atrial fibrillation and
chronic heart failure, that may require concomitant drug treatment. In this non-systematic review we want to summarize the pleiotropic antithrombotic effects and the underlying mechanism of the most common drugs used in cardiovascular patients, foremost statins, antihypertensive, antiarrhythmic and antidiabetic drugs.

2. Methods
A comprehensive literature search was conducted, mainly using PubMed database. The search terms were adjusted due to our clinical experience and included the terms: "pleiotropic antithrombotic effects, antithrombotic effects or anti platelet effects" and "statins, antihypertensive drugs, antiarrhythmic drugs, antiarrhythmics or antidiabetic drugs". Moreover, we enlarged our findings upon articles that gave further insight in the underlying biochemical mechanisms of the pleiotropic antithrombotic effects. This non-systematical review makes no pretense to completeness and ought to provide an overview about the preexisting data.

3. Statins
The JUPITER trial, published more than 10 years ago, was a primary prevention trial in mid-aged individuals without previously known cardiovascular disease and normal LDL-cholesterol levels of less than 130 mg/dL but an elevated high sensitive CRP above 2 mg/L. 17,802 participants were enrolled and randomized to rosvastatin versus placebo. As a first result the occurrence of a first cardiovascular event, as a mixed endpoint of nonfatal myocardial infarction, nonfatal stroke, hospitalization for unstable angina, an arterial revascularization procedure, or confirmed death from cardiovascular causes, was significantly reduced by rosvastatin when the study was terminated after a median follow-up of 1.9 years [1]. The marked reduction of the mixed endpoint was driven by fewer atherothrombotic complications. Interestingly, the study revealed that lowering of high sensitive CRP seemed to convey additional and partially independent benefits over lowering LDL-cholesterol alone [2]. Another analysis of the same study focused on venous thrombosis and pulmonary embolism and showed that rosvastatin reduced venous thromboembolic events as well. The venous thrombotic events occurred independently of cardiovascular atherothrombotic complications, i.e. patients that did not experience a reduction in cardiovascular events still experienced less venous thromboembolic events [3]. The reduction of venous thromboembolic events and the effect of lowering high sensitive CRP suggest that there is more to statins than just cholesterol lowering. There are several potential mechanisms by which statins influence pathways of platelet aggregation and blood coagulation.

Statins were reported to interfere with coagulation factors. They can suppress the release of tissue factor (TF) from inflammatory and endothelial cells [4], which may otherwise trigger the extrinsic blood coagulation cascade by forming the active complex of TF and factor VIIa [5]. A randomized trial including 24 patients showed that treatment with simvastatin for eight weeks significantly decreases TF activity compared to dietary restriction alone [4]. Another study demonstrated that atorvastatin reduces the expression of CD40 ligand (CD40L) on platelets and circulating levels of soluble (s) CD40L, independent of its lipid lowering effects [6]. CD40L is a member of the tumor necrosis factor family, expressed by various cell types such as endothelial cells (EC), smooth muscle cells, and platelets, and is associated with
proinflammatory and prothrombotic effects [7]. CD40L stimulates thrombus formation partly via CD40-mediated induction of TF generation. Thus, atorvastatin, by reducing CD40L expression, may inhibit TF release and activation of the extrinsic coagulation pathway. A torvastatin and simvastatin were reported to reduce prothrombin (FII) and thrombin (FIIa) formation, respectively [6,8]. Additionally, statins stimulate thrombomodulin (TM) expression on EC that scavenges FIIa, resulting in inhibition of fibrin formation and platelet activation [9]. The authors suggested that statins inhibit protein prenylation of Rho GTPases, which then lose their inhibitory effect on TM generation in EC. Besides affecting fibrin formation, TM-bound FIIa may activate protein C which in turn degrades procoagulant factor Va (FVa) and factor VIIIa (FVIIIa). In summary, statins impact thrombus formation by interfering with the production and function of various coagulation factors.

Another possible mechanism that may be relevant for the antithrombotic effects of statins is the suppression of platelet-leukocyte interaction. For example, CD40 expressing immune cells can interact with CD40L-expressing platelets, whereby statins reduce CD40L expression on platelets [7]. In line with this hypothesis, patients admitted for acute coronary syndrome (ACS) demonstrate less circulating platelet-leukocyte aggregates in the first 24h after early administration of rosuvastatin [10]. Whether reduced platelet-leukocyte aggregates are linked to antithrombotic effects remains to be determined.

Statins may have an early antioxidant effect independent of cholesterol-lowering by downregulating NADPH oxidase 2 [12]. Thromboxane A2 (TxA2) activates platelets in an auto- and paracrine fashion, and is inactivated within seconds through hydrolyzation to thromboxane B2 (TxB2) [13]. Pignatelli et al. [12] measured reduced level of the TxB2 metabolite in blood as a surrogate for reduced TxA2 production, proposing that statins may inhibit phospholipase A2 upstream the arachidonic acid pathway.

Another mechanism by which statins are postulated to impair platelet aggregation is the increase in intraplatelet nitric oxide (NO). NO is produced by endothelial nitric oxide synthase (eNOS) in EC and platelets. NO activates the soluble guanylate cyclase (sGC) that increases cyclic guanosine monophosphate (cGMP) levels and intracellular calcium (Ca2+) levels, resulting in inhibition of platelet adhesion [14]. Daily intake of atorvastatin for three weeks stimulates eNOS production in human platelets [15]. Taken together, statins suppress platelet activation via multiple pathways (Figure 1).

Intracellular oxidative stress activates platelets. Accordingly, deficiency in glutathione peroxidase-3, an enzyme that scavenges reactive oxygen species, leads to increased platelet-dependent thrombosis [11].
Figure 1: Schematic overview of the stimulatory (blue) and inhibitory (red) pleiotropic antithrombotic effects of statins (adapted from Bäuml and Hilgendorf [50])

4. Antihypertensive drugs

**RAS inhibitors:** Arterial hypertension represents one of the major cardiovascular risk factors. Several studies have demonstrated that some of the daily used antihypertensive drugs may exert pleiotropic antithrombotic effects. Initially, the focus was on drugs interfering with the renin-angiotensin system (RAS). Ridker et al. [16] injected angiotensin II into healthy volunteers to explore links between hypertension and thrombotic complications. He observed that increasing doses of angiotensin II elevated plasma levels of plasminogen activator inhibitor (PAI-1) while decreasing free tissue plasminogen activator (tPA). PAI-1 forms an inactivating complex with tPA, thereby preventing the generation of fibrinolytic plasmin [17]. Elevated levels of PAI-1 in blood have been linked to thromboembolic events [18,19]. Interestingly, angiotensin converting enzyme (ACE)-inhibitors and angiotensin II receptor (AT1)-blockers (ARB) led to an increase in tPA and a decrease in PAI-1 production [20]. Angiotensin II stimulates PAI-1 release from EC, counteracted by ACE-inhibitors and ARB [21]. In addition, ACE-inhibitors increase bradykinin levels that stimulate tPA release [22]. The inhibitory effect of ACE-inhibitors on PAI-1 activity has been confirmed by an analysis of a subgroup of 120 post-MI patients in the HEART study treated with ramipril. Paired blood samples from the index event and at 2 weeks follow-up were available. Compared with the placebo group, PAI-1 antigen and PAI-1 activity levels were significantly lower at day 14 in the group of ramipril-treated patients. In contrast, plasma tPA levels were not significantly different between the placebo-treated and ramipril-treated groups [23] (Figure 2).

Similar to statins, blocking of TF release from monocytes, and stimulation of endothelial and
platelet NO release have been observed with ACE inhibitors and ARB, resulting in suppressed platelet activation [24]. Activation of eNOS was induced dose-dependently by losartan and valsartan, but not by candesartan suggesting that this mode of action may not apply to all ARB [25].

Some ARB seem to inhibit the thromboxane receptor, directly. Guerra-Cuesta et al. [26] described that losartan reduces the binding capacity of TxA₂ to its receptor on platelets. This effect seems to be dose dependent, however, supraphysiological doses of losartan need to be applied. Clinical data of the randomized ‘Valsartan Inhibits Platelets’-trial showed that valsartan exerts platelet inhibitory effects already after 5 weeks of treatment independent of the doses used (80, 160 or 320 mg). This observation was based on multiple platelet surface receptors being downregulated upon valsartan therapy, in particular GPIIb and activated GP IIb/IIIa, P-selectin and CD40-ligand. The authors hypothesize that these changes result from valsartan modulating complex endothelial-platelet interactions, rather than affecting a single receptor or pathway [27] (Figure 2).

**Calcium antagonists:** Another frequently used class of antihypertensive drugs is dihydropyridine calcium antagonists. A systematic review including 22 studies showed in 2006 that calcium antagonists significantly enhanced fibrinolysis caused by an increase in tPA antigen level and a decrease in PAI-1 [28] (Figure 2). The reservation must be made, however, that fibrinolytic activity of dihydropyridine calcium antagonists is weak compared to the commonly used fibrinolytic drugs like urokinase or recombinant tissue plasminogen activator (rtPA), and is only functional in patients with impaired fibrinolysis but not in healthy individuals.

**sGC stimulators:** Of note, a novel drug class of direct, NO-independent sGC stimulators is making its way into heart failure treatment. Vericiguat was reported to reduce cardiovascular death and hospitalization for heart failure events in patients with heart failure with reduced ejection fraction (HFrEF) [29]. A similar drug, riociguat, currently marketed for treatment of pulmonary arterial hypertension, was evaluated for its effects on platelets. While direct stimulation of sGC by riociguat did inhibit activation of isolated platelets (Figure 2), it failed to do so in whole blood which was speculated to result from binding to serum proteins. Whether this applies to vericiguat remains to be tested [30].
Figure 2: Schematic overview of the stimulatory (blue) and inhibitory (red) pleiotropic antithrombotic effects of antihypertensive drugs (adapted from Bäuml and Hilgendorf[50]).

5. Antiarrhythmic drugs

Class II agents: β-blockers are frequently used in coronary heart disease, heart failure and arrhythmia. By blocking beta adrenergic receptors, they lower heart rates and decrease blood pressure and myocardial oxygen demand. In addition, β-blockers may also affect platelet aggregation. A meta-analysis of 31 clinical studies in which a total of 454 patients were treated with β-blockers, and platelet aggregation was tested ex vivo, described a modest antithrombotic effect [31]. The lipophilic, nonselective β-blocker carvedilol showed a bigger impact on platelet aggregation than selective β-blockers. Yet, the underlying mechanism responsible for the antithrombotic effect of β-blockers is likely indirect. There are many more α2-receptors expressed on the platelet surface than β2-receptors. Probably, decreased generation of plasma catecholamines due to β-blocker therapy, leads to lower activation of α2-receptors, increased intraplatelet levels of cyclic adenosine monophosphate (cAMP) and increased Ca^{2+} availability, resulting in reduced platelet activation [32] (Figure 3). The hypothesis is supported by the observation that nonselective β-blockers are more efficient in reducing sympathetic activity and in suppressing platelet aggregation [33]. Nevertheless, the antithrombotic effects of β-blockers seem rather modest and of subordinate importance, clinically.

Class III agents: Amiodarone is a commonly used antiarrhythmic drug in patients with atrial fibrillation to establish and sustain sinus rhythm, or to suppress ventricular arrhythmias. A meta-analysis of 13
randomized studies in 6,553 patients with symptomatic, compensated, congestive heart failure with a mean left-ventricular ejection fraction of 31% or prior acute myocardial infarction showed that amiodarone reduced the incidence of sudden cardiac death (SCD) [34]. Whether these beneficial outcomes are solely based on preventing arrhythmias, or whether prevention of lethal thromboembolic complications may have contributed, is unknown. Beside its multiple unfavorable side effects, there is preclinical evidence that amiodarone prevents thrombus formation.

Vascular TF activity and total platelet numbers were reduced in a mouse model of photochemical injury in vivo upon amiodarone treatment [35] (Figure 3). In human blood (ex vivo), dronedarone was shown to exert direct anticoagulant and antithrombotic effects [36]. These antithrombotic pleiotropic effects may contribute to the reduction of stroke and transient ischemic attacks in patients with persistent or paroxysmal atrial fibrillation under treatment with class III antiarrhythmic agents [37].

Figure 3: Schematic overview of the inhibitory (red) pleiotropic antithrombotic effects of antiarrhythmic drugs (adapted from Bäuml and Hilgendorf60)

6. Antidiabetic drugs

Biguanides: Metformin is the most widely used oral antidiabetic drug in type II diabetes mellitus (T2D). It is speculated that metformin may improve mortality by reducing diabetes-associated thrombotic events [38,39]. In metformin-treated rats, experimentally induced arterial and venous thrombus formation were significantly reduced, and the susceptibility to pulmonary embolism markedly decreased [40]. Mechanistically, reduced platelet prothrombinase activity, and αIIbβ3 and P-selectin expressions were made responsible. Moreover, metformin increases the phosphorylation of eNOS in EC resulting in an enhanced production of platelet-inhibiting NO [41].
Metformin reduces PAI-1 levels that are elevated in patients with T2D to balance out prothrombotic and fibrinolytic activity [42] (Figure 4). In the 10-year follow-up of the United Kingdom Prospective Diabetes Study (UKPDS), long-term use of metformin was associated with reductions in myocardial infarctions independent of glycemic control in obese diabetics [43]. Interestingly, metformin-treated mice did not show any bleeding complications and the bleeding time did not change significantly suggesting that metformin therapy might be a safe option of antithrombotic treatment [40].

**GLP-1 receptor agonists and DPP-4 inhibitors:**

Novel antidiabetic drugs, such as glucagon-like peptide 1 receptor agonists (GLP-1RA), carry now a Class IA recommendation for treating patients with T2D and predominantly atherosclerotic cardiovascular disease according to the 2019 ESC guidelines [44]. GLP-1RA were shown to lower the rates of atherothrombotic complications such as myocardial infarction and stroke in T2D patients with coronary heart disease. Regarding potential antithrombotic actions of GLP-1RA, liraglutide reduced PAI-1 plasma levels in clinical trials in contrast to other antidiabetic drug classes [45] (Figure 4). Experimental studies showed that exenatide inhibits platelet aggregation in murine and human blood samples stimulated with thrombin, ADP or collagen [46]. Using a laser injury mouse model and intravital microscopy, the authors showed that exenatide treatment markedly reduced thrombus formation. In contrast, glucagon-like peptide 1 receptor-deficient mice featured increased platelet aggregability, suggesting that the drugs act via their canonical receptor. Binding of GLP-1 analogs to the GLP-1 receptor increases intraplatelet cAMP which controls a large number of distinct platelet functions and suppresses platelet activity [47]. Similar effects have been reported for dipeptidyl peptidase 4 (DPP-4) inhibitors such as linagliptin or sitagliptin, that inhibit the degradation of endogenous GLP-1, lending further support to the atheroprotective ancillary actions of these drugs beyond their effects on glycemic control [48,49] (Figure 4).
Figure 4: Schematic overview of the stimulatory (blue) and inhibitory (red) pleiotropic antithrombotic effects of antidiabetic drugs (adapted from Bäuml and Hilgendorf)7.

7. Concluding remarks
Cardiovascular disease and atherothrombotic complications are among the leading causes of morbidity and mortality in the world. Thus, cardiovascular drugs represent highly effective options to treat millions of patients. Beside their main mode of action, they exert ancillary effects such as pleiotropic antithrombotic effects. While not increasing the risk of bleeding, these pleiotropic antithrombotic effects may contribute to the overall cardiovascular benefits of statins and certain antihypertensive, antiarrhythmic and antidiabetic drugs. Clinicians and scientists may thus want to consider the ancillary effects in their daily practice and research.

Conflict of Interest: The authors declare no relevant conflicts of interest.

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Abbreviations
ACE - Angiotensin converting enzyme
ACS - Acute coronary syndrome
ARB - AT1-blockers
ASCVD - Atherosclerotic cardiovascular disease
AT1 - Angiotensin II receptor
Ca²⁺ - Calcium
CD40L - CD40 ligand
cAMP - Cyclic adenosine monophosphate
cGMP - Cyclic guanosine monophosphate
DPP-4 - Dipeptidyl peptidase 4
EC - Endothelial cells
eNOS - Endothelial nitric oxide synthase
FII - Prothrombin
FIIa - Thrombin
FVa - Factor Va
FVIIIa - Factor VIIIa
GLP-1R - Glucagon-like peptide 1 receptor
GLP-1RA - Glucagon-like peptide 1 receptor agonists
HFrEF - Heart failure with reduced ejection fraction
NO - Nitric oxide
PAI-1 - Plasminogen activator inhibitor
RAS - Renin-angiotensin system
rtPA - Recombinant tissue plasminogen activator
SCD - Sudden cardiac death
sGC - Soluble guanylate cyclase
T2D - Type II diabetes mellitus
TF - Tissue factor
TM - Thrombomodulin
tPA - Tissue plasminogen activator
TxA₂ - Thromboxane A₂
TxB₂ - Thromboxane B₂
UKPDS - United Kingdom Prospective Diabetes Study

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