Antiinflammatory effects of aspirin in ACS: relevant to its cardiocoronary actions?

Thomas Hohlfeld; Karsten Schrör
Institut für Pharmakologie und Klinische Pharmakologie, Heinrich-Heine-Universität Düsseldorf, Düsseldorf, Germany

Summary
Vascular injury in acute coronary syndromes (ACS) involves a complex cross-talk between inflammatory mediators, platelets and thrombosis, where the interaction between platelets and coagulation factors (e.g. thrombin) is a central link between thrombosis and inflammation. In ACS, aspirin at antiplatelet doses exhibits anti-inflammatory effects as seen from the decrease in inflammation markers such as CRP, M-CSF, MCP-1 and others. These actions probably occur subsequent to inhibition of platelet COX-1-dependent thromboxane formation and its action as a multipotent autocrine and paracrine agent. This likely involves inhibition of thrombin formation as well as inhibition of secondary pro-inflammatory mediators, such as sphingosine-1-phosphate. Experimental and limited clinical data additionally suggest antiinflammatory effects of aspirin independent of its antiplatelet action. For example, aspirin at antiplatelet doses might acetylate COX-2 in vascular cells, directing the activity of the enzyme into a 15-lipoxygenase which by transcellular metabolism results in the formation of 15-epi-lipoxin (‘aspirin-triggered lipoxin’), an antiinflammatory mediator. Furthermore, aspirin stimulates ENOS via lysine-acetylation, eventually resulting in induction of heme oxygenase (HO-1), which improves the antioxidative potential of vascular cells. All of these effects have been seen at antiplatelet doses of 100–300 mg/day, equivalent to peak plasma levels of 10–30 µM. Many more potentially antiinflammatory mechanisms of aspirin have been described, mostly salicylate-related, at low to medium millimolar concentrations and, therefore, are of minor clinical interest. Altogether, there is a wealth of data supporting antiinflammatory effects of aspirin in ACS, but studies generating direct evidence for antiinflammatory effects in ACS remain to be done.

Keywords
Aspirin, platelet pharmacology, thromboxane, inflammatory mediators, thrombin

Introduction
The key event which defines an acute coronary syndrome (ACS) is the formation of an intracellular thrombus, usually resulting from platelet activation in large coronary arteries at the site of a ruptured plaque with physical lumen occlusion by thrombosis. Conventionally, the favourable effect of aspirin in acute atherothrombosis is the prevention of platelet activation via suppression of thromboxane (TX) A2 formation (1). This is explained by the irreversible inactivation of platelet COX-1 by transfer of an acetyl moiety from aspirin to a serine residue next to the substrate binding site, which irreversibly prevents binding of arachidonic acid and its conversion to PGG2H2. In platelets, PGH2 is further converted by TX synthase to TXA2 which potently amplifies platelet activation. This mechanism of platelet inhibition is unique to aspirin and the pharmacological reason for the synergistic effects of aspirin and other antiplatelet drugs in ACS.

TXA2 is a multipotent chemical mediator. Thus, the inhibition of its formation likely eliminates any further autocrine and paracrine actions of TXA2 on cells in the neighbourhood. For example, aspirin in antiplatelet doses has been shown to inhibit leukocyte accumulation at a local inflammatory site and to stimulate the production of secondary anti-inflammatory mediators (lipoxins) in man (2). There is also a role for inhibition by aspirin of platelet-mediated recruitment of inflammatory cells and soluble inflammatory mediators, such as C-reactive protein (CRP), in a broad range of cardiovascular diseases (3–6). By comparison with other antiplatelet agents, it is not entirely clear whether this interaction with inflammatory mediators is a direct, TXA2-mediated effect or indirectly mediated via the antiplatelet activity (7).

Platelets also contain several TXA2-sensitive and potentially proinflammatory platelet storage products whose release might be inhibited by aspirin. For example, sphingosine-1-phosphate (SIP) is an inflammatory mediator stored in platelets, which is released in a strictly aspirin-sensitive manner (8). SIP stimulates monocyte migration and other proinflammatory cell functions. We have recently shown that aspirin reduces thrombin-induced platelet secretion of SIP (9). The clinical significance of such complex
interactions between platelet-derived mediators, TXA₂ and other cells, i.e. so-called “heterotypic” platelet functions (10), may be considerable but is incompletely understood yet.

Hence, platelet activation and secretion result in platelet-mediated inflammatory conditions (11) and the antiplatelet effects of aspirin also involve anti-inflammatory actions. This article summarises the role of inflammation in ACS, outlines actual concepts on how aspirin may modulate inflammation in acute coronary syndromes and gives an overview on more recently discovered and less well established pharmacological concepts on the action of aspirin in ACS.

**Inflammation in ACS**

Among the most prominent events precipitating coronary artery thrombosis in ACS is the formation and rupture of a coronary artery plaque. Recruitment of inflammatory cells, large lipid-rich plaque cores and thin fibrous caps predict a high-risk of plaque rupture and subsequent ACS. In each of these conditions, thrombotic as well as inflammatory pathways, are involved. Analyses of the culprit atherosclerotic lesions after myocardial infarction demonstrated that plaque erosion and rupture are essentially determined by inflammatory processes in the vascular wall (12).

At all stages of atheroma formation monocytes and lymphocytes adhere to the endothelium, transmigrate, become activated and release their activation products. This involves cytokines, such as interleukins, CD40 ligand (CD40L), tumour necrosis factor (TNF)-α and others, which enable activation of more leukocytes. Activation of endothelial cells leads to expression of chemokines and endothelial adhesion molecules causing further recruitment of inflammatory cells into the subendothelium (13). Activated cells in the plaque proliferate, mature into macrophages, accumulate lipoproteins and form foam cells, the characteristic elements of atherosclerotic lesions. Intercellular matrix degradation by proteolytic enzymes (e.g. matrix metalloproteases, MMP) degrade collagen and elastin of the fibrous cap, rendering it more fragile for sudden rupture and arterial thrombus formation. Overall, the extent of plaque inflammation is correlated with the severity of coronary stenosis and the risk of plaque rupture (14).

**Platelets and thrombin as a major source of vascular inflammation**

Experimental and clinical experience suggest that platelets adhere at high shear stress to the injured or inflamed vascular endothelium initially via endothelial (P-selectin, von Willebrand factor (vWF)) and platelet surface proteins (PSGL-1, GP1bα). This is followed by more stable adhesion via platelet αIIb/β3 integrin, fibronogen and vascular αvβ3 and involves receptor interactions mediated by selectins, integrins and immunoglobulin-like receptors, which generate activation signals in platelets and endothelial cells (15). Activated platelets expose adhesion proteins (fibrinogen, vWF, P-selectin), chemoattractants (e.g., platelet factor 4, sphingosine-1-phosphate), cytokines (e.g., CD40L) and coagulatory proteins such as tissue factor (TF), of which some are expressed from pre-formed RNA (16). By these mechanisms, platelets play a crucial role in vascular inflammation, including foam cell and plaque formation, with a strong relation to the risk for acute coronary thrombotic events (15, 17).

Subsequent to plaque rupture in ACS, TF becomes immediately available and allows for thrombin generation which subsequently ‘explodes’ in the propagation phase of coagulation. ▶Table 1 summarises the central role of thrombin in atherothrombosis and vascular inflammation. Thrombin is the most potent platelet

<table>
<thead>
<tr>
<th>Aspirin</th>
<th>Clopidogrel</th>
<th>Aspirin plus clopidogrel</th>
<th>Aspirin plus prasugrel</th>
<th>GP IIb/IIIa inhibitors</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD40L</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>CRP</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td>X</td>
</tr>
<tr>
<td>IL-6</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>MAC-1</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M-CSF</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PLA formation</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>P-selectin</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>TF-PCA</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

X indicates modification of formation or expression of marker in separate studies. Direct comparisons between different antiplatelet treatments are missing. CD40L, CD40 ligand; CRP, C-reactive protein; GP, glycoprotein; IL-6, interleukin-6; MAC-1, macrophage-1-antigen (CD11b/CD18); MCP-1, monocyte chemoattractant protein-1, M-CSF, macrophage colony stimulating factor; PLA, platelet-leucocyte aggregates; TF-PCA, tissue factor procoagulant activity; TNF-α, tumour necrosis factor α.

**Table 1: Effects of aspirin and other antiplatelet agents, as well as their combinations, on inflammatory markers in atherothrombotic disease (7).**
stimulus. It is generated at the surface of activated platelets and amplifies its formation by positive feed-back to coagulation factor V. Notably, the amount of thrombin formed is in the nanomolar range (18), i.e. several orders of magnitude higher than necessary for stimulation of its major platelet receptor PAR-1, as opposed to the concentrations required for fibrinogen cleavage (19). Activated platelets then provide the matrix for further bulk thrombin formation and there is feedback to TXA$_2$ production, which acts as both an initiating and amplifying event. Generation of thrombin also plays, in addition to thrombosis, a key role in the progression of vascular inflammation and atherosclerosis (20). Thrombin receptors especially of the PAR-1 and PAR-4 subtypes on platelets and vascular cells have been implicated in the development of atherosclerosis (21). Thrombin is an important regulator of vascular inflammation by stimulating the release of interleukins (IL-6, IL-8) and macrophage migration inhibitory factor (MIF) from endothelial and smooth muscle cells (21, 22). Moreover, thrombin-activated platelets induce the secretion of MCP-1 and ICAM-1 from endothelial cells and enhance endothelial vimentin receptor ($\alpha$, $\beta$) surface expression in an IL-1-dependent manner (23). Vascular inflammation likely induces a chronic hyperinflammatory state (Figure 1). Indeed, markers of thrombin formation, such as thrombin fragment 1+2 and thrombin-antithrombin complexes, are increased in ACS (24) and remain elevated for up to 1 year (25, 26).

Taken together, vessel wall injury in ACS involves an extensive cross-talk between mediators of inflammation, coagulation, platelets and the vessel wall, where platelets and coagulation factors (e.g. thrombin) form a link between vascular injury, inflammation and thrombosis.

Antiinflammatory effects of aspirin by inhibition of platelet-derived proinflammatory products

Since activated platelets are potent sources of cytokines, adhesion molecules and inflammatory lipid mediators, one may expect that antiplatelet drugs, including aspirin, exert an antiinflammatory effect in settings of thrombosis-associated inflammation by inhibition of release of inflammatory products.

TXA$_2$ is released in large amount by activated platelets and extraplatelet sources (e.g. monocytes/macrophages) and acts in an autocrine and paracrine manner on G protein-coupled TXA$_2$ (TP) receptors signalling via phospholipase C and other cellular targets. Via this pathway, TXA$_2$ not only activates platelets but also monocytes and macrophages, endothelial cells and vascular smooth muscle cells and thereby stimulates vascular inflammation (27). The expression of TXA$_2$ synthase is increased in atherosclerotic lesions and associated with thrombotic events (28). TXA$_2$ and other TP receptor agonists (e.g. isoprostanes) increase endothelial expression of proinflammatory markers (29), while TP receptor antagonists inhibit proinflammatory transcription via TNF-$\alpha$ (30).

The importance of TXA$_2$ in atherothrombosis is underlined by studies demonstrating that enhanced urinary excretion of thromboxane metabolites in aspirin-treated individuals is associated with an increased risk of ACS (31, 32). Hence, available data suggest that aspirin exerts antiinflammatory effects in settings of thrombosis-associated inflammation by its primary mechanism of action, i.e. irreversible inhibition of TXA$_2$ synthesis, mediated by inhibition of platelet COX-1 (Figure 2).
TXA₂ also mediates inflammatory responses indirectly by regulating the release of other pro-inflammatory products. One example is the immune-modulating lipid mediator sphingosine-1-phosphate (S1P) (Figure 2). S1P is stored in the platelet cytosol released in large amounts upon appropriate platelet activation, for example by thrombin. S1P has been identified as an important link between platelet activation and the immune system (33). It also synergises with thrombin by upregulating tissue factor in endothelial cells (34), which may be detrimental in a prothrombotic environment such as ACS. Moreover, S1P upregulates thrombin receptor (PAR-4) expression of monocytes, enhances thrombin-mediated monocyte migration and promotes in concert with other chemokines the migration of monocytes towards sites of vascular injury (35–37).

Previous work from our laboratory has demonstrated that S1P release from platelets involves TX-dependent signalling, since an inhibitor of TX synthase as well as a TP receptor antagonist effectively inhibited platelet S1P release (35). In line with this, aspirin also inhibited thrombin-induced release of S1P from platelets by inhibition of platelet COX-2 in a strictly thromboxane-dependent manner in both healthy volunteers as well as patients with ACS (9). Clearly, the biological targets of S1P are complex and more research is needed to characterise the role of S1P in ACS and the consequences of the inhibition by aspirin. Recently, circulating myeloid-related protein 8/14, another circulating platelet secretion product which regulates inflammation and predicts cardiovascular events, has been shown to be substantially reduced by low dose aspirin in ACS (38).

As mentioned above, ACS is associated with an explosion of thrombin formation during the first hours after the acute event. This thrombin-induced platelet activation is ‘resistant’ against ADP-antagonists and probably also aspirin (39, 40). However, aspirin in contrast to ADP antagonists was found to inhibit (retard) clotting-associated thrombin formation \textit{ex vivo} (41–44) and acted synergistically in this respect with low-dose rivaroxaban in an experimental setting (45). This action is lost in case of ‘aspirin resistance’ (46), suggesting that aspirin-induced inhibition of thrombin formation might be a consequence of its – thromboxane-mediated – antiplatelet effect. Indeed, thrombin formation in ACS has been shown to be aspirin-sensitive and may well contribute to its clinical efficacy in dual antiplatelet therapy in ACS beyond the ‘pure’ inhibition of platelet aggregate formation (47).

**Effects of aspirin on circulating markers of inflammation in chronic cardiovascular disease and ACS**

Several studies have shown that aspirin, beyond inhibition of TXA₂-mediated platelet activation, also decreases circulating
levels of inflammatory biomarkers. One example is C-reactive protein (CRP), an acute phase reactant and frequently used biomarker of systemic inflammation. CRP is released in inflammatory states and is increased in the plasma of patients with acute myocardial ischaemia (4, 48) and myocardial infarction (49, 50). There is a positive relationship between CRP and troponin-I as a marker of infarct size (4). CRP predicts recurrent ischaemia among subjects with ACS (51) and an increased incidence of cerebrovascular events (52). Despite its immunological effects, it is controversial whether CRP should be regarded in this context as a pro-inflammatory protein or merely a biomarker of inflammation (53).

Among the first studies to show an association of low dose aspirin with serum levels of CRP was a sub-analysis of the Physicians’ Health Study, a large prospective, randomised, double-blind placebo-controlled trial in healthy physicians which analysed primary prevention of cardiovascular events by 325 mg aspirin every other day (5). The substudy compared plasma CRP in the baseline blood samples of 543 participants who experienced myocardial infarction, stroke or venous thrombosis during the trial, as well as in the same number of matched controls without events. There was a proportional association of baseline CRP with the rate of vascular events (Figure 3, grey bars), suggesting a close association of baseline CRP with the rate of cardiovascular events. Moreover, the prevention of myocardial infarction by aspirin (red bars in Figure 3) was most extensive (56% risk reduction) in the quartile with the highest baseline CRP, while an only moderate, non-significant trend of cardiovascular prevention (~14%) was seen in the quartile with lowest CRP. Thus, the benefit by aspirin was highly dependent on baseline CRP in an almost linear manner across the quartiles. The finding was robust after adjustment of numerous other risk factors. These data suggest that the benefit of aspirin may have, at least partly, been dependent on an antiinflammatory action.

It should be mentioned that, vice versa, acute inflammation may counteract the antiplatelet action of aspirin through mechanisms such as upregulation of COX-1-independent platelet activation (thrombin, CRP) or increasing thromboxane synthesis via vascular or platelet COX-2, which is less sensitive to aspirin than COX-1, the isoform normally present in platelets. The issue of aspirin nonresponsiveness (or “aspirin resistance”) and its impact on clinical outcome is beyond the focus of this article (for recent reviews see [54, 55]).

A number of subsequent studies aimed to further examine the antiinflammatory effect of aspirin by examination of CRP in different patient populations (3, 56–68). These could not confirm this finding in healthy volunteers or subjects with low cardiovascular risk (56–61, 63) but observed a significant fall in (elevated) CRP in patients with stable coronary artery disease and ACS (3, 64–68). One study (62) found a decrease of CRP by aspirin in a subgroup of patients with coronary disease receiving statins, suggesting that the known antiinflammatory effects of statins (69, 70) may demask or synergise with an antiinflammatory action of aspirin.

Other inflammatory markers, such as IL-6, TNF-α, sCD40L and myeloperoxidase and macrophage colony stimulating factor (M-CSF), which are elevated in the plasma or upregulated at the site of coronary occlusion, are also associated with an increased risk of myocardial infarction (71, 72) or all-cause mortality (73–75). Some of these are lowered by aspirin, including CRP, IL-6, MCP-1 and M-CSF (3, 64, 66, 76) at antiplatelet doses of 100–300 mg/day. Table 1 summarises effects of aspirin alone and in combination with other antiplatelet drugs on selected inflammatory mediators reported in the literature (7). The profile differs significantly among antiplatelet drugs, reflecting different modes of action and antiplatelet activities. Unfortunately, direct comparisons between different antiplatelet treatments are missing.

Indirect evidence for aspirin effects on inflammatory markers also comes from increased IL-6, RANTES and CRP levels in the plasma of patients with symptomatic CAD, who showed an elevated arachidonic acid-induced whole blood aggregation indicating an individual impairment of the platelet response to aspirin (77). Another study observed a positive association of the proinflammatory mediators IL-6, IFN-γ and interferon-inducible protein-10 in conjunction with lower levels of the antiinflammatory cytokines IL-4 and IL-10 in subjects with a decreased platelet responsiveness to aspirin (78).

Platelet-independent antiinflammatory mechanisms of aspirin

There is evidence from numerous – mostly experimental – studies that aspirin also exerts TXA2-independent effects at different steps of vascular inflammation. Table 2 gives an overview on the broad range of available studies and experimental settings. Of particular interest are those which reported effects at therapeutically relevant (low micromolar) aspirin concentrations.

Vascular inflammation and angioplasty are accompanied with the production of the pro-resolving eicosanoid lipoxins (LX) A4 and B4 by a COX-2- and 12/15-lipoxygenase-dependent transcellular metabolism (79, 80). Aspirin targets both COX-1 and COX-2 in vascular and inflammatory cells, though the latter at somewhat higher concentrations. While COX-1 acetylation inhibits enzyme...
activity, acetylation of COX-2 alters its catalytic mechanism, directing enzymatic activity of endothelial cells and monocytes towards formation of 15R-hydroxyeicosatetraenoic acid (15R-HETE) (▶Figure 2). This is unique to aspirin and not seen with conventional non-steroidal anti-inflammatory drugs (NSAIDs) or coxibs. 15R-HETE is converted via neutrophil and monocyte 5-lipoxygenase to 15-epi-LXA$_4$ (‘aspirin-triggered lipoxin’, ATL), which modulates and resolves vascular inflammatory responses by stimulating G protein coupled FPR2/ALX receptors on different types of inflammatory cells such as neutrophils, monocytes, macrophages and T cells (79). For example, a recent study showed in a mouse model of mesenterial ischaemia that aspirin, by triggering 15-epi-LXA$_4$ formation, potently prevented neutrophil adhesion and transmigration (81). Of note, 15-epi-LXA$_4$ is triggered by aspirin in healthy humans at an oral dose of 81 mg/day (82) and a similar dose (75 mg/day) reduced neutrophil and macrophage accumulation in skin lesions independently of NF-$\kappa$B-regulated gene expression and local prostaglandin synthesis (2). Non-aspirin NSAIDs and coxibs slightly but significantly increase the risk of atherothrombosis in subjects with advanced cardiovascular disease by inhibiting the endothelial (largely COX-2-dependent) synthesis of antithrombotic prostaglandins, such as PGI$_2$. It has been debated whether this may also apply to aspirin (‘aspirin dilemma’). However, COX-2 inhibition by aspirin in nucleated cells at antiplatelet doses is only transient (hours) and incomplete and thus differs fundamentally from the irreversible COX-1 inhibition in platelets (83, 84). These unique pharmacological features of

### Table 2: Selected studies demonstrating molecular mechanisms of aspirin potentially important for antiinflammatory actions in cardiovascular disease.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Experimental model</th>
<th>Aspirin dose or concentration</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylation of COX-2 resulting in transcellular increase of 15-epi-LXA$_4$</td>
<td>Downregulation of vascular neutrophil adhesion and transmigration in a mouse model of mesenterial ischaemia/reperfusion</td>
<td>30–100 mg/kg i.p.</td>
<td>(81)</td>
</tr>
<tr>
<td>Acetylation of eNOS, increase of endothelial NO synthesis</td>
<td>Porcine coronary arteries and immortalised human umbilical vein endothelial cells</td>
<td>10 nmol/l – 10 µmol/l</td>
<td>(86)</td>
</tr>
<tr>
<td>eNOS-dependent induction of heme oxygenase (HO-1) protein and activity</td>
<td>Human umbilical vein endothelial cells</td>
<td>30–300 µmol/l</td>
<td>(88)</td>
</tr>
<tr>
<td>Inhibition of IKK-$\beta$ and NFkB signalling</td>
<td>IKK-$\beta$-transfected COS-cells and HeLa-cells</td>
<td>0.01–10 mmol/l (also salicylate)</td>
<td>(97)</td>
</tr>
<tr>
<td>Suppression of fractalkine/CX3CL1 in atherosclerotic lesions with reduction of plaque size</td>
<td>ApoE-gene knockout mice</td>
<td>58 mg/kg/day orally</td>
<td>(98)</td>
</tr>
<tr>
<td>Reduction of aortic NFkB activity with decrease in sICAM-1, MCP-1, TNF-$\alpha$ and IL-12p40</td>
<td>LDL-receptor-deficient mice on high-fat diet</td>
<td>~3 mg/kg/day orally</td>
<td>(99)</td>
</tr>
<tr>
<td>Suppression of COX-2 gene transcription via C/EBP$\beta$ binding to COX-2 promoter</td>
<td>Serum-deprived quiescent human fibroblasts</td>
<td>10 µmol/l</td>
<td>(100)</td>
</tr>
<tr>
<td>Inhibition of superoxide formation, p38MAPK activation and ox-LDL-mediated expression of LOX-1 and MMP-1</td>
<td>Human coronary endothelial cells</td>
<td>1–5 mmol/l</td>
<td>(101)</td>
</tr>
<tr>
<td>Enhancement of PKB/Akt phosphorylation and improvement of insulin sensitivity</td>
<td>Liver/muscle of Zucker fa/fa rats</td>
<td>120 mg/kg/day i.v. (also salicylate)</td>
<td>(102)</td>
</tr>
<tr>
<td>Inhibition of NADPH oxidase activity with improvement of endothelial function</td>
<td>Normo- and hypertensive rats</td>
<td>10–100 mg/kg/day (12 days)</td>
<td>(103)</td>
</tr>
<tr>
<td>Impairment of proteasome function with accumulation of ubiquitinylated proteins (Bax, IκB-α, p53, p27kip1)</td>
<td>Mouse neuro 2a cells</td>
<td>1–50 mmol/l</td>
<td>(104)</td>
</tr>
</tbody>
</table>

Figure 4: Concentration-dependent increase of NO formation (amperometric sensor) by aspirin and APHS (o-(acetoxy-phenyl)hept-2-ynyl sulfide) from porcine coronary arteries. Aspirin and its derivative APHS increase NO formation, likely due to eNOS acetylation by transfer of the acetyl residue (gray) attached to the phenol. Na$^+$ salicylate was largely ineffective. Data from (86).
aspirin may explain the therapeutic benefit seen with this compound but not with NSAIDs and coxibs.

Aspirin is also known to alter nitric oxide (NO) synthesis, which besides its known effects on the vasculature plays a prominent role in inflammation (85). Endothelial and platelet NO synthase (eNOS) are stimulated by aspirin (Figure 2) (86, 87). This effect is independent of COX-1 or COX-2 inhibition and seen at concentrations as low as 10 µM (Figure 4) and is probably caused by lysine acetylation of eNOS (1). Moreover, aspirin-induced NO was associated with an induction of heme oxygenase (HO) –1, a downstream target of NO relevant for the adaptive response to oxidative stress and inflammation (88). Subsequent work examined serum HO-1 in patients with chronic stable coronary disease, showing a 70% increase of HO-1 within 12 weeks of aspirin treatment at doses of 81 mg/day and higher, as well as a 40% decrease of asymmetric dimethyl arginine (ADMA), an endogenous NO synthase inhibitor involved in endothelial dysfunction (89, 90). Accordingly, low dose aspirin improved endothelial function in patients with elevated cardiovascular risk (91). While HO-1 as well as its reaction products (carbon monoxide and bilirubin) may decrease myocardial injury and improve post-infarct ventricular remodelling (92), it remains to be shown whether this mechanism is involved in prevention of ACS by aspirin.

Numerous other TXA2-independent effects of aspirin on mediators or signalling of inflammatory responses have been reported, such as prevention of nuclear translocation of the pro-inflammatory transcription factor NFkB (93), decreased expression of inflammatory target genes of NFkB (94), suppression of endothelial upregulation of VCAM-1 and E-selectin (95) and others (Table 2). Such effects were not specific for aspirin but were also seen with salicylate, suggesting acetylation-independent mechanisms. Most of such data was gathered experimentally with millimolar concentrations, largely exceeding the plasma concentrations achieved clinically by antiplatelet doses of aspirin (96). Hence, it is unlikely that such effects of aspirin are relevant for treatment or prevention of ACS.

**Conclusions**

Numerous in vitro and in vivo studies suggest that aspirin not only inhibits platelet aggregation, but exerts a spectrum of additional pharmacological effects targeting a broad spectrum of inflammatory pathways of potential importance in vascular inflammation. These actions may or may not involve platelets as primary targets. It is likely that thrombin formation, either thromboxane-induced or thromboxane-independent, represents a central link between the platelet/coagulation and inflammatory pathways. Several studies also demonstrated direct anti-inflammatory actions of aspirin, such as generation of anti-inflammatory mediators (e.g. ATL) and antioxidant actions due to eNOS activation with subsequent upregulation of HO-1. Importantly, such effects occur at antiplatelet doses of aspirin. The spectrum of experimental data, however, contrasts with sparse data from clinical studies. Indeed, much of the available data comes from research with platelets or cells derived from healthy subjects or animal experimentation and the spectrum of aspirin effects might differ in human disease. Thus, clinical research with aspirin such as prospective comparisons versus placebo or alternative treatments would be desirable, but may be problematic or even unfeasible in ACS because aspirin is an indispensable part of the standard of care. It is also difficult to separate actions of aspirin from those of ADP agonists in dual antiplatelet therapy of ACS as mandated by current guidelines. With this in mind and taking into account that aspirin has been demonstrated to reduce inflammatory markers in patients at advanced stages of cardiovascular disease, it nevertheless appears justified to conclude that anti-inflammatory effects of aspirin are likely to contribute to its clinical efficacy in ACS.

**Conflicts of interest**

None declared.

**References**


Valgimigli M, et al. Prasugrel versus tirofiban bolus with or without short post- bolus infusion with or without concomitant prasugrel administration in patients with myocardial infarction undergoing coronary stenting: the FABULOUS PRO (Facilitation through Aggrastat by DrOpping or Shortening Infusion Line in pa-tients undergoing ST-segment elevation myocardial infarction compared to or on top of PrAsugrel given at loading dose(s)). JACC Cardiovasc Interv 2012; 5: 268–277.


