Prothrombotic Potential of NSAID in Ischemic Heart Diesase

Florian Krötz[a,b,*], Nicole Hellwig[b], Thomas M. Schiele[a], Volker Klauss[a] and Hae-Young Sohn[a]

[a]*Institute of Cardiology and* [b]*Physiology, Medical Policlinic, Ludwig-Maximilians-University, Munich;* [a]*Ziemssenstr. 1, 80336 Munich;* [b]*Schillerstr. 44, 80336 Munich, Germany*

Abstract: Non-steroidal anti-inflammatory drugs (NSAID) target the enzyme cyclooxygenase (COX) thus affording relieve from pain, inflammation or fever. As COX-dependently formed prostanoids not only mediate signals involved in inflammation and pain, but also regulate important physiological cardiovascular functions, some NSAID have recently been reported to be associated with arterial thrombosis or hypertension. This is in contrast to the well-known antiplatelet effects of low-dose aspirin, but in coherence with the specific effects of some NSAID on prostanoid formation in the vasculature. A correlation between the intake of selective inhibitors of the cyclooxygenase 2 (COX-2) isoform and atherothrombotic events has recently been established. Large retrospective analyses of clinical data have repeatedly shown this effect and in some cases have also observed potential hazards for other, rather non-selective NSAID. This review evaluates potential prothrombotic effects of NSAID in vascular ischemic disease in comparison to low-dose aspirin and selective COX-2 inhibitors and discusses pathophysiological backgrounds for such observations.

INTRODUCTION

Ever since the introduction of aspirin to medical therapy, COX enzymes have represented a prominent target for pharmacotherapy. As aspirin is an effective inhibitor of platelet activation, the appraisal that other non-steroidal anti-inflammatory drugs (NSAID) may also exert antithrombotic effects *in vivo* is common.

However, COX inhibitors prevent the formation of several prostanoids with relevance for vascular biology and they are thus involved in multiple physiological and pathophysiological cellular processes. More than one isoform of COX exists and their tissue-specific expression patterns, spatial and functional association with enzymes involved in further degradation of the major COX product and the specific pharmacological properties of COX inhibitors make the ultimate physiological effects of these drugs a difficult pharmacological question.

On a molecular basis, the COX enzyme is a homodimeric protein that generates prostaglandin G_2, (PGG_2) from arachidonic acid (AA). PGG_2 is immediately biotransformed by the same enzymatic complex to the cyclic endoperoxide prostaglandin H_2 (PGH_2) [1-3]. This product accounts for the often used and scientifically more correct name of COX, prostaglandin H synthase (PGHS) [4, 5]. There are several isoforms of COX existing in humans. COX-1 can be detected in virtually any tissue, and is many times expressed at constant levels throughout the cell cycle, which has resulted in its characterisation as a "housekeeping" enzyme [6, 7]. COX-2, in contrast to COX-1, is normally described as an enzyme inducible after stimulation with numerous inflammatory agents [6-8]. Remarkably, in vascular endothelial cells, COX-2 is constitutively expressed and prostanoids formed from it participate either in the paracrine or autocrine regulation of vessel function [3, 6]. A third isoform called COX-3 has recently been discussed to be the target of acetaminophen [9, 10], but its existence has not been described in humans so far.

As PGH_2 is the main product of PGHS (and thus COX) activity, further enzymatic complexes that are functionally linked to PGHS may be specifically expressed in a tissue and are necessary to generate the ultimate products of interest for human physiology, the prostaglandins. Important examples are thromboxane A_2 (TxA_2) synthase, the main prostanoid synthase in platelets, or prostaglandin I_2 synthase (prostacyclin synthase, PGI_2 synthase) [5], which is e.g. expressed in endothelial cells, where it is also linked to COX-2 [11].

With respect to vascular biology and thrombosis, prostacyclin (prostaglandin I_2, PGI_2) and thromboxane A_2 (TxA_2) are the most relevant prostanoids. Whereas TxA_2 synthase is the main prostanoid synthase in platelets, PGI_2 synthase seems to be of high importance in endothelial cells, where it is linked to COX-1 and COX-2 [11, 12]. The actual tissue specific expression of different COX isoforms, their association with tissue-dependent prostanoid-synthases, and the resulting balance between the two most important prostanoids for vascular homeostasis, TxA_2 and PGI_2, thus is of crucial importance for atherothrombosis and for the question of a pro- or antithrombotic effect of any COX inhibiting substance.

Due to their differential inhibitory effects on the formation of different prostanoids that mediate physiological as well as inflammatory signals COX inhibitors not only mediate desired effects, but also alter physiological processes. The question of effects of NSAID on atherothrombosis other than inhibitory ones has first been brought about by clinical findings of prothrombotic effects of specific inhibitors of the COX-2 [3, 13, 14]. These drugs had initially promised to largely improve pharmacotherapy, because they potentially reduced undesired side effects such as gastrotoxicity, but

*Address correspondence to this author at the Cardiology, Medical Policlinic, Ludwig-Maximilians-University, Ziemssenstr.1, 80336 Munich, Germany; Tel: +49-89-218075-384; Fax: +49-89-218075-378,
E-mail: florian.kroetz@med.uni-muenchen.de

1389-5575/06 $50.00+.00

they have later been suggested to enhance the risk of atherothrombotic events *in vivo*. As a consequence, some of these substances had been withdrawn from global markets by their manufacturers. As the mechanisms of their potential prothrombotic action become ever clearer, it needs to be questioned, whether some rather non-selective COX inhibitors (non-steroidal anti-inflammatory drugs, NSAID), would also have a prothrombotic effect *in vivo*, or whether they are indeed antithrombotic, as could be assumed in analogy to the effects of aspirin. In the following, experimental and clinical evidence for pro- or antithrombotic effects of low-dose aspirin, selective COX-2 inhibitors, and of non-selective NSAID will be reviewed in more detail to elucidate the rationale why NSAID may not only inhibit, but also trigger atherothrombotic events *in vivo*.

LOW-DOSE ASPIRIN

Inhibition of platelet aggregation by aspirin has been suggested to be of use in the prevention of coronary thrombosis in the 1950's already [15] and was first described in the 1960's by Weiss and colleagues [16]. Acetyl salicylic acid (ASA, aspirin) inhibits the activation of COX by irreversibly acetylating a serine residue inside the hydrophobic channel formed by COX [17]. It is about 170-fold more effective in inhibiting COX-1 than in inhibiting COX-2 [7]. The latter and the irreversibility of the acetylation at the serine residues lead to nearly complete inhibition of COX in anucleate, cell-like structures like platelets which can only ineffectively regenerate the enzyme [18], but an insufficient inhibition of COX activity in cells that are able to resynthesize the enzyme. When low-dose aspirin is delivered daily, there is an accumulation of the drug in platelets that is accompanied by a marginal effect on COX in other tissues. Thus, data at hand suggest that the inhibitory effects of aspirin on platelet TxA_2 synthesis clearly dominate the inhibitory – and potentially prothrombotic - effects on endothelial PGI_2 synthesis [19].

Rapid absorption after oral delivery, rapid clearance from the systemic circulation and irreversible binding to platelet COX-1 lead to inhibition of platelet function as soon as 1 hour after delivery of non-coated, oral aspirin [19]. The inhibitory effect is increased by repetitive dosage and reaches a maximum after about 5 days of treatment [20, 21]. In addition to its effects on platelets, there is experimental evidence that aspirin prevents development of cardiovascular disease by improving endothelial dysfunction in atherosclerotic vessels [22], or by preventing oxidation of LDL [23].

Clinically, low-dose aspirin is effective in the prevention of arterial thrombosis in various disease settings such as the acute treatment of acute coronary syndromes [24-26], as well as in the secondary or the primary prevention of myocardial infarction or cerebrovascular thrombotic disease [27-32]. Of note, a recently published prospective study about primary prevention in women failed to show that aspirin affects the risk of myocardial infarction or death from cardiovascular causes although it lowered the risk of stroke in these patients [33]. Today, aspirin, when applied daily at a low-dose, represents a cheap and safe strategy to prevent myocardial infarction or stroke in patients at risk and is thus the most established strategy of secondary prevention of atherothrombotic disease.

SELECTIVE COX-2 INHIBITORS

Selective COX-2 inhibitors (also called Coxibs) have been accused to promote atherothrombosis, as recent studies have indicated that Coxibs even have the capacity of triggering atherothrombosis due to their somewhat specific inhibition of the production of PGI_2 released from the vascular endothelium [34-36, 36]. Evidence for this can be derived from experimental and clinical studies, which clearly show, that there is enhanced *in vivo* platelet activation during selective inhibition of COX-2 [37], that arterial thrombus formation is accelerated when COX-2 is inhibited selectively [38, 39] and that the latter is not the case when non-selective inhibition of COX is performed. Clinically, selective COX-2 inhibitors have been suspected to increase the risk for vascular thrombosis ever since the VIGOR trial [13]. However, it was only in 2004 that these effects could be confirmed by large clinical surveys, which resulted in the withdrawal of rofecoxib from global markets [40, 41]. Following this a plethora of data attributing a prothrombotic effect of selective COX-2 inhibitors has been published [14, 40, 42-46]. With regard to celecoxib, the second selective COX-2 inhibitor that had been marketed, there have been incidental reports about thrombotic events [47], which could not be confirmed by larger studies [41, 48, 49]. Nevertheless, the manufacturer of celecoxib had warned of potential cardiovascular atherothrombotic side effects in December 2004 because preliminary results from the PreSAP and AFC trials gave evidence for dose- and time of intake related increases in cardiovascular events due to celecoxib [46, 50]. Fewer data are at hand for the newer Coxibs such as lumiracoxib, etoricoxib, valdecoxib or parecoxib (a prodrug of valdecoxib for i.v. use). Although many of the clinical trials that investigated their efficiencies in rheumatic disease did not show enhanced rates of atherothrombotic events [51-54], in a study in patients undergoing coronary artery bypass grafting, the use of parecoxib/ valdecoxib has been reported to increase all cause serious adverse effects in 2003 already [43, 55]. The most likely pathophysiological cause of enhanced thrombosis rates during selective COX-2 inhibitor intake is their influence on availability of the most relevant prostanoids for vascular function and thrombosis, PGI_2 and TxA_2 [2]. Numerous experimental studies in animals or humans have shown that selective inhibition of COX-2 reduces levels of PGI_2 metabolites without having any or just a little effect on TxA_2 production [38, 56-63]. More importantly, several studies have also shown now that this leads to an enhanced risk of atherothrombosis *in vivo* [38, 39, 64-66]. Although non of these studies suggest that Coxibs may cause spontaneous thrombosis, further studies in mice with a deletion of either the PGI_2 receptor (IP receptor) or of the TxA_2 receptor (TP receptor) support this assumption that selective COX-2 inhibitors enhance platelet activation and thus are able to trigger the onset of thrombotic events [67]. In summary, it is now well-documented experimentally that the balance between vascular levels of TxA_2 and PGI_2 is involved in the prothrombotic effects of selective COX-2 inhibitors. Nonetheless, there are several important remaining questions, such as the question whether simultaneous delivery of low-dose aspirin can reverse this prothrombotic effect and the important question, whether the rather non-selective COX inhibitors, which are often subsumed in the drug group called

NSAID rather have pro- or antithrombotic effects in patients [68].

NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

The recognition of prothrombotic side effects of selective inhibitors of COX-2 has also brought about the question, whether traditional NSAID – with similarity to aspirin – exert antihrombotic effects or tend to have effects on atherothrombosis that resemble those exerted by the Coxibs, which would mean that they are potentially prothrombotic [69-71]. Whereas repeated delivery of low-doses of aspirin has little effect on immediate or long-term COX activity in the endothelium due to the above mentioned transcriptional novel synthesis of COX, endothelial COX-2 still has some – although limited - sensitivity to the drug [19, 72]. Therefore, high doses of aspirin could have similar effects on endothelial PGI_2-synthesis as on platelet TxA_2 synthesis, thus theoretically exerting antithrombotic as well as prothrombotic effects.

In general, the group of NSAID, drugs that inhibit COX rather non-specifically, includes derivates of acetic acid, proprionic acid, pyrazole and other chemically distinct substances. They are competitive inhibitors of AA binding at PGHS. Alike low-dose aspirin, NSAID can inhibit platelet activation – especially in *ex vivo* assays of isolated platelet preparations- because of their inhibitory effect on platelet formation of TxA_2. Based on the pharmamcokinetics of NSAID and their effects on the formation on TxA_2 and PGI_2 *in vivo* these drugs are likely not to have the ability of preventing thrombosis *in vivo*, although several studies indicate that they may inhibit platelet activation *in vitro*. In contrast to aspirin, the binding of NSAID to COX is reversible, and their inhibitory effect on TxA_2 production may only last shortly [37]. The limited time-span and the reversibility of NSAID binding to COX may explain reports of NSAID not being as effective antiplatelet agents as low-dose aspirin is [47, 73]. In addition, most non-aspirin NSAID lack sufficient specifity for a COX isoform, which is why they also inhibit the formation of vascular COX products other than platelet TxA_2, such as preventing PGI_2 formation *in vivo* [37]. Due to this, NSAID may still be effective in blocking platelet activation *ex vivo* and *in vitro* and are used in numerous research projects to prevent e.g. platelet aggregation in platelet-rich plasma (PRP) or whole blood assays. In such experimental setups, PGI_2 produced by COX in the vascular wall is not present any more. Thus, when a NSAID is added to a full-blood or platelet-rich plasma preparation, it will merely affect platelet TxA_2 formation and therefore have an antithrombotic effect, although this would not be the case if endothelium-derived PGI_2 would be present. This has in some cases led to the false assumption that findings from such *in vitro* studies could be used to argue in favour of a cardioprotective effect of some NSAID such as naproxen [37, 74]. Many other features of non-aspirin NSAID make them inadequate choices for the goal of efficient prevention of arterial thrombosis. There often is a short plasma half-life time, not allowing for sufficient concentrations of the NSAID to guarantee a stable and long-lasting COX inhibition [3]. It has repeatedly been investigated whether high doses of aspirin are more effective in reducing cardiovascular mortality in secondary prevention than low doses of aspirin. According to the hypothesis of the balance between PGI_2 and TxA_2 being the most important function of a pro- or antithrombotic net effect of a drug *in vivo*, one would tend to suggest that a chronic high dosage of aspirin is rather less effective antithrombotically than a low dosage. However, in contrast to its effects on TxA_2 released from platelets, chronic administration of high dose aspirin dose not guarantee a long-lasting suppression of prostanoids released from inflammatory cells [19]. Most trials aiming at investigating the dose-effect relationship of aspirin with respect to cardiovascular mortality show that the protective effect starts at a low dose of usually 75mg/d and is sustained up to higher doses of even 1.500mg/d (for review see [19]). Nevertheless, data from the Antiplatelet Trialists Collaboration suggest that to some degree there may be an inverse relationship between an increasing dose of aspirin and its antithrombotic efficiency [75]. Nonetheless, this unclear question highlights the fact that the prostanoid balance model can merely be a simplified mechanistic model for the effect of an NSAID on atherothrombosis in patients.

Independent of high dose aspirin, most NSAID certainly should not be a choice for therapeutic prevention of atherothrombosis, although, in some clinical situations, patients that are on NSAID treatment, may be at an increased risk of bleeding, especially when they are combined with oral anticoagulants like coumadin [76]. Above their influence on TxA_2 and PGI_2 levels in the vasculature NSAID may interfere with plasma-protein binding of certain drugs or they may modulate hepatic metabolism of oral anticoagulants [77]. Recently, NSAID have also been discussed to potentially prevent the antiplatelet effects of low-dose aspirin. This has been described as "aspirin resistance". When measuring serum thromboxane B2 levels as an index of platelet TxA_2 formation in patients receiving chronic low-dose aspirin therapy, one study found that simultaneous delivery of ibuprofen in a single or in repeated doses, reversed aspirin-dependent maximum inhibition of TxB_2 formation and significantly prevented the effect of aspirin on platelet aggregation [78]. However, other NSAID like acetaminophen or diclofenac had no such effect in this study. Meanwhile, several clinical studies found that there was indeed an increased atherothrombotic risk when ibuprofen and aspirin was taken concomitantly [79, 80].

Nevertheless, a potentially cardioprotective, antiplatelet efficiency of naproxen – the non-selective NSAID with which rofecoxib had been compared in the VIGOR study - has soon been discussed after the results from VIGOR had been published. This study had first been interpreted in a way that there would not be an increased thrombotic risk for rofecoxib, but that the NSAID it was compared with, naproxen, would exert cardioprotective, antithrombotic affects, similar as low-dose aspirin, which could not be taken by the patients of the VIGOR study. Indeed, a number of observational studies have subsequently attributed cardioprotective properties to naproxen [69, 70, 80]. In one of these retrospective analyses in 4,425 patients hospitalised for myocardial infarction, only naproxen, but none of the other non aspirin NSAID were associated with a reduced risk of myocardial infraction [70]. Cardioprotective effects of naproxen were noted more than once following VIGOR, but only infrequently for other non-aspirin NSAID such as ibu-

profen [80], and these findings could not be confirmed by all authors [81, 82]. A retrospective survey of more than 33.000 NSAID users also did not show cardioprotective effects of naproxen [81]. Similar conclusions were drawn after an overview of the Tennessee Medicaid programme of more than 180.000 NSAID [82].

In contrast to these observations, today some data even attribute an increased risk of atherothrombosis to NSAID. Intriguingly, in a study using naproxen, in patients at risk of developing Alzheimer's disease, this drug apparently increased the risk of cardiovascular events, which is why it was halted by the US National Institute on aging (see: http://www.fda.gov/bbs/topics/news/2004/NEW01148.html). Also the large Kaiser-Permanente Survey found a small increase in risk of myocardial infarction among naproxen users compared with users of other NSAID [83]. Other retrospective studies even suggested an increased risk for the general group of conventional NSAID, and drugs like diclofenac or ibuprofen have been among the substances for which respective trends to enhanced cardiovascular complication rates or even statistical correlations have been established [46, 84-86]. However, these results have to be regarded as preliminary information, which should prompt research efforts on controlled investigation of the actual effect of chronic administration of a NSAID on cardiovascular thrombotic events.

SUMMARY

When discussing the effects of COX inhibition on atherothrombosis, a clear look at the specific drug is needed in order to actually decide whether it may be used as a platelet inhibitor or whether it rather exerts prothrombotic properties. As a mnemonic orientation guide, three groups of COX inhibitors may be distinguished because of their mechanistic influence on the formation of TxA_2 and PGI_2, the two vascular prostanoids, which are most relevant to platelet activity and thus, atherothrombosis.

First, low-dose aspirin at daily dosage, is a quite selective inhibitor of COX-1. It thus nearly exclusively prevents TxA_2 production and has antiplatelet effects, which are well documented in terms of atherothrombosis prevention in clinical settings. It clearly is the only good choice among COX inhibitors for therapeutic prevention of atherothrombosis.

Second, selective inhibitors of COX-2 most likely have an intrinsic prothrombotic effect *in vivo*, because they may suppress endothelium-derived PGI_2 as an important antithrombotic agent in arterial vessels. This may trigger the onset of atherothrombotic complications, especially when a Coxib is administrated to a patient at cardiovascular risk. However, a clear-cut correlation between atherothrombotic risk and a specific Coxib can so far only be assumed for rofecoxib. The prothrombotic potential of other Coxibs is not unlikely but should not be assumed unless prospective studies suggest so. Regardless, data from retrospective studies, which had caused public concern about prothrombotic side effects of these drugs, have already prompted the withdrawal of many Coxibs from global markets. Taken together, selective COX-2 inhibitors that are still marketed should be used with great care in patients at risk for cardiovascular atherothrombosis. In the future, the concurrent use of low-dose aspirin, may be sufficient to alleviate the prothrombotic hazard of a Coxib [68]. Whether this combination still offers the advantage of decreased gastrointestinal toxicity, however, remains to be shown. Potentially a combination with a non-COX inhibiting antiplatelet drug may be of advantage, such as with clopidogrel. The latter, however, may not be cost-effective any more.

As a third group, other, rather non-selective NSAID likely do not induce sufficient antithrombotic effects, because they theoretically are not specific for either COX-1 or COX-2. Moreover, usually these drugs are not delivered at doses and timing intervals that result in stable inhibition of platelet TxA_2 without affecting endothelial PGI_2, which may on the one hand cause increases in bleeding times under specific circumstances, but on the other hand still not afford sufficient antithrombotic protection. In accordance with this, clinical experiences support the view that NSAID should not be used as antithrombotic substances in the prevention of cardiovascular disease instead of aspirin. Clinical data about potential antithrombotic properties of naproxen or other NSAID are controversial and limited to retrospective studies. Of note, some recent retrospective analyses even support the view that some NSAID – with similarity to Coxibs – may even increase cardiovascular hazard by increasing atherothrombotic event rates. Prospective studies addressing the topic are lacking. However, interactions between non-aspirin NSAID and low-dose aspirin may potentially decrease antithrombotic properties of low-dose aspirin.

REFERENCES

[1] Warner, T.D.; Mitchell, J.A. *FASEB J.*, **2004,** *7*, 790.
[2] Davidge, S.T. *Circ. Res.*, **2001,** *8*, 650.
[3] FitzGerald, G.A. *Nat. Rev. Drug Discov.*, **2003,** *11*, 879.
[4] Smith, W.L.; DeWitt, D.L.; Garavito, R.M. *Ann. Rev. Biochem.*, **2000**, *69*, 145.
[5] Helliwell, R.J.; Adams, L.F.; Mitchell, M.D. *Prostaglandins Leukot. Essent. Fatty Acids*, **2004,** *2*, 101.
[6] Hinz, B.; Brune, K. *J. Pharmacol. Exp. Ther.*, **2002,** *2*, 367.
[7] Vane, J.R.; Bakhle, Y.S.; Botting, R.M. *Ann. Rev. Pharmacol. Toxicol.*, **1998**, *38*, 97.
[8] Hinz, B.; Brune, K.; Pahl, A. *Biochem. Biophys. Res. Commun.*, **2000,** *3*, 790.
[9] Chandrasekharan, N.V.; Dai, H.; Roos, K.L.; Evanson, N.K.; Tomsik, J.; Elton, T.S.; Simmons, D.L. *Proc. Natl. Acad. Sci. USA*, **2002,** *21*, 13926.
[10] Schwab, J.M.; Schluesener, H.J.; Laufer, S. *Lancet*, **2003,** *9362*, 981.
[11] Liou, J.Y.; Shyue, S.K.; Tsai, M.J.; Chung, C.L.; Chu, K.Y.; Wu, K.K. *J. Biol. Chem.*, **2000,** *20*, 15314.
[12] Wu, K.K.; Liou, J.Y. *Biochem. Biophys. Res. Commun.*, **2005,** *1*, 45.
[13] Bombardier, C.; Laine, L.; Reicin, A.; Shapiro, D.; Burgos-Vargas, R.; Davis, B.; Day, R.; Ferraz, M.B.; Hawkey, C.J.; Hochberg, M.C.; Kvien, T.K.; Schnitzer, T.J. *N. Engl. J. Med.*, **2000,** *21*, 1520.
[14] Krotz, F.; Schiele, T.M.; Klauss, V.; Sohn, H.Y. *J. Vasc. Res.*, **2005,** *4*, 312.
[15] CRAVEN, L.L. *Miss. Valley. Med. J.*, **1953,** *1*, 38.
[16] Weiss, H.J.; Aledort, L.M.; Kochwa, S. *J. Clin. Invest.*, **1968,** *9*, 2169.
[17] Loll, P.J.; Picot, D.; Garavito, R.M. *Nat. Struct. Biol.*, **1995,** *8*, 637.
[18] Evangelista, V.; Manarini, S.; Di Santo, A.; Capone, M.L.; Ricciotti, E.; Di Francesco, L.; Tacconelli, S.; Sacchetti, A.; D'Angelo, S.; Scilimati, A.; Sciulli, M.G.; Patrignani, P. *Circ. Res.*, **2006,** *5*, 593.
[19] Patrono, C.; Coller, B.; Dalen, J.E.; FitzGerald, G.A.; Fuster, V.; Gent, M.; Hirsh, J.; Roth, G. *Chest*, **2001,** *1 Suppl*, 39S.
[20] Patrignani, P.; Filabozzi, P.; Patrono, C. *J. Clin. Invest.*, **1982,** *6*, 1366.
[21] Cipollone, F.; Patrignani, P.; Greco, A.; Panara, M.R.; Padovano, R.; Cuccurullo, F.; Patrono, C.; Rebuzzi, A.G.; Liuzzo, G.; Quaranta, G.; Maseri, A. *Circulation*, **1997,** *4*, 1109.

[22] Husain, S.; Andrews, N.P.; Mulcahy, D.; Panza, J.A.; Quyyumi, A.A. *Circulation*, **1998,** *8*, 716.
[23] Steer, K.A.; Wallace, T.M.; Bolton, C.H.; Hartog, M. *Heart*, **1997,** *4*, 333.
[24] Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction: ISIS-2. ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. *Lancet*, **1988,** *8607*, 349.
[25] Nyman, I.; Larsson, H.; Wallentin, L. *Lancet*, **1992,** *8818*, 497.
[26] Wallentin, L.C. *J. Am. Coll. Cardiol.*, **1991,** *7*, 1587.
[27] Awtry, E.H.; Loscalzo, J. *Circulation*, **2000,** *10*, 1206.
[28] Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*, **2002,** *7329*, 71.
[29] Physician's health study: aspirin and primary prevention of coronary heart disease. *N. Engl. J. Med.*, **1989,** *26*, 1825.
[30] Peto, R.; Gray, R.; Collins, R.; Wheatley, K.; Hennekens, C.; Jamrozik, K.; Warlow, C.; Hafner, B.; Thompson, E.; Norton, S. *Br. Med. J. (Clin. Res. Ed.)*, **1988,** *6618*, 313.
[31] Thrombosis prevention trial: randomised trial of low-intensity oral anticoagulation with warfarin and low-dose aspirin in the primary prevention of ischaemic heart disease in men at increased risk. The Medical Research Council's General Practice Research Framework. *Lancet*, **1998,** *9098*, 233.
[32] Manson, J.E.; Stampfer, M.J.; Colditz, G.A.; Willett, W.C.; Rosner, B.; Speizer, F.E.; Hennekens, C.H. *JAMA*, **1991,** *4*, 521.
[33] Ridker, P.M.; Cook, N.R.; Lee, I.M.; Gordon, D.; Gaziano, J.M.; Manson, J.E.; Hennekens, C.H.; Buring, J.E. *N. Engl. J. Med.*, **2005,** *13*, 1293.
[34] Mukherjee, D. *Biochem. Pharmacol.*, **2002,** *5*, 817.
[35] Bing, R.J.; Lomnicka, M. *J. Am. Coll. Cardiol.*, **2002,** *3*, 521.
[36] Mukherjee, D.; Nissen, S.E.; Topol, E.J. *JAMA*, **2001,** *8*, 954.
[37] Leese, P.T.; Hubbard, R.C.; Karim, A.; Isakson, P.C.; Yu, S.S.; Geis, G.S. *J. Clin. Pharmacol.*, **2000,** *2*, 124.
[38] Buerkle, M.A.; Lehrer, S.; Sohn, H.Y.; Conzen, P.; Pohl, U.; Krotz, F. *Circulation*, **2004,** *14*, 2053.
[39] Hennan, J.K.; Huang, J.; Barrett, T.D.; Driscoll, E.M.; Willens, D.E.; Park, A.M.; Crofford, L.J.; Lucchesi, B.R. *Circulation*, **2001,** *7*, 820.
[40] Juni, P.; Nartey, L.; Reichenbach, S.; Sterchi, R.; Dieppe, P.A.; Egger, M. *Lancet*, **2004,** *9450*, 2021.
[41] Solomon, D.H.; Schneeweiss, S.; Glynn, R.J.; Kiyota, Y.; Levin, R.; Mogun, H.; Avorn, J. *Circulation*, **2004,** *17*, 2068.
[42] Choi, H.K.; Seeger, J.D.; Kuntz, K.M. *Am. J. Med.*, **2004,** *9*, 621.
[43] Nussmeier, N.A.; Whelton, A.A.; Brown, M.T.; Langford, R.M.; Hoeft, A.; Parlow, J.L.; Boyce, S.W.; Verburg, K.M. *N. Engl. J. Med.*, **2005,** 1081.
[44] Solomon, S.D.; McMurray, J.J.; Pfeffer, M.A.; Wittes, J.; Fowler, R.; Finn, P.; Anderson, W.F.; Zauber, A.; Hawk, E.; Bertagnolli, M. *N. Engl. J. Med.*, **2005,** 1071.
[45] Bresalier, R.S.; Sandler, R.S.; Quan, H.; Bolognese, J.A.; Oxenius, B.; Horgan, K.; Lines, C.; Riddell, R.; Morton, D.; Lanas, A.; Konstam, M.A.; Baron, J.A. *N. Engl. J. Med.*, **2005,** 1092.
[46] Johnsen, S.P.; Larsson, H.; Tarone, R.E.; McLaughlin, J.K.; Norgard, B.; Friis, S.; Sorensen, H.T. *Arch. Intern. Med.*, **2005,** *9*, 978.
[47] Crofford, L.J.; Oates, J.C.; McCune, W.J.; Gupta, S.; Kaplan, M.J.; Catella-Lawson, F.; Morrow, J.D.; McDonagh, K.T.; Schmaier, A.H. *Arthritis Rheum.*, **2000,** *8*, 1891.
[48] White, W.B.; Faich, G.; Borer, J.S.; Makuch, R.W. *Am. J. Cardiol.*, **2003,** *4*, 411.
[49] Moore, R.A.; Derry, S.; Makinson, G.T.; McQuay, H.J. *Arthritis Res. Ther.*, **2005,** *3*, R644.
[50] European Medicines Agency. *EMEA/214027/2004*, **2004,** 1.
[51] White, W.B.; Strand, V.; Roberts, R.; Whelton, A. *Am. J. Ther.*, **2004,** *4*, 244.
[52] Farkouh, M.E.; Kirshner, H.; Harrington, R.A.; Ruland, S.; Verheugt, F.W.; Schnitzer, T.J.; Burmester, G.R.; Mysler, E.; Hochberg, M.C.; Doherty, M.; Ehrsam, E.; Gitton, X.; Krammer, G.; Mellein, B.; Gimona, A.; Matchaba, P.; Hawkey, C.J.; Chesebro, J.H. *Lancet*, **2004,** *9435*, 675.
[53] Hunt, R.H.; Harper, S.; Watson, D.J.; Yu, C.; Quan, H.; Lee, M.; Evans, J.K.; Oxenius, B. *Am. J. Gastroenterol.*, **2003,** *8*, 1725.
[54] Pallay, R.M.; Seger, W.; Adler, J.L.; Ettlinger, R.E.; Quaidoo, E.A.; Lipetz, R.; O'Brien, K.; Mucciola, L.; Skalky, C.S.; Petruschke, R.A.; Bohidar, N.R.; Geba, G.P. *Scand. J. Rheumatol.*, **2004,** *4*, 257.
[55] Ott, E.; Nussmeier, N.A.; Duke, P.C.; Feneck, R.O.; Alston, R.P.; Snabes, M.C.; Hubbard, R.C.; Hsu, P.H.; Saidman, L.J.; Mangano, D.T. *J. Thorac. Cardiovasc. Surg.*, **2003,** *6*, 1481.
[56] Catella-Lawson, F.; McAdam, B.; Morrison, B.W.; Kapoor, S.; Kujubu, D.; Antes, L.; Lasseter, K.C.; Quan, H.; Gertz, B.J.; FitzGerald, G.A. *J. Pharmacol. Exp. Ther.*, **1999,** *2*, 735.
[57] McAdam, B.F.; Catella-Lawson, F.; Mardini, I.A.; Kapoor, S.; Lawson, J.A.; FitzGerald, G.A. *Proc. Natl. Acad. Sci. USA*, **1999,** *1*, 272.
[58] McAdam, B.F.; Mardini, I.A.; Habib, A.; Burke, A.; Lawson, J.A.; Kapoor, S.; FitzGerald, G.A. *J. Clin. Invest.*, **2000,** *10*, 1473.
[59] Qi, Z.; Hao, C.M.; Langenbach, R.I.; Breyer, R.M.; Redha, R.; Morrow, J.D.; Breyer, M.D. *J. Clin. Invest.*, **2002,** *1*, 61.
[60] Pidgeon, G.P.; Tamosiuniene, R.; Chen, G.; Leonard, I.; Belton, O.; Bradford, A.; Fitzgerald, D.J. *Circulation*, **2004,** *17*, 2701.
[61] Burleigh, M.E.; Babaev, V.R.; Oates, J.A.; Harris, R.C.; Gautam, S.; Riendeau, D.; Marnett, L.J.; Morrow, J.D.; Fazio, S.; Linton, M.F. *Circulation*, **2002,** *15*, 1816.
[62] Widlansky, M.E.; Price, D.T.; Gokce, N.; Eberhardt, R.T.; Duffy, S.J.; Holbrook, M.; Maxwell, C.; Palmisano, J.; Keaney, J.F., Jr.; Morrow, J.D.; Vita, J.A. *Hypertension*, **2003,** *3*, 310.
[63] Llinas, M.T.; Lopez, R.; Rodriguez, F.; Roig, F.; Salazar, F.J. *Am. J. Physiol. Renal. Physiol.*, **2001,** *5*, F975.
[64] Kearney, D.; Byrne, A.; Crean, P.; Cox, D.; Fitzgerald, D.J. *J. Am. Coll. Cardiol.*, **2004,** *4*, 526.
[65] Belton, O.A.; Duffy, A.; Toomey, S.; Fitzgerald, D.J. *Circulation*, **2003,** *24*, 3017.
[66] Belton, O.; Byrne, D.; Kearney, D.; Leahy, A.; Fitzgerald, D.J. *Circulation*, **2000,** *8*, 840.
[67] Cheng, Y.; Austin, S.C.; Rocca, B.; Koller, B.H.; Coffman, T.M.; Grosser, T.; Lawson, J.A.; FitzGerald, G.A. *Science*, **2002,** *5567*, 539.
[68] Grosser, T.; Fries, S.; FitzGerald, G.A. *J. Clin. Invest.*, **2006,** *1*, 4.
[69] Rahme, E.; Pilote, L.; LeLorier, J. *Arch. Intern. Med.*, **2002,** *10*, 1111.
[70] Solomon, D.H.; Glynn, R.J.; Levin, R.; Avorn, J. *Arch. Intern. Med.*, **2002,** *10*, 1099.
[71] Mukherjee, D.; Nissen, S.E.; Topol, E.J. *Arch. Intern. Med.*, **2002,** *22*, 2637.
[72] Patrono, C.; Patrignani, P.; Garcia Rodriguez, L.A. *J. Clin. Invest*, **2001,** *1*, 7.
[73] Capone, M.L.; Tacconelli, S.; Sciulli, M.G.; Grana, M.; Ricciotti, E.; Minuz, P.; Di Gregorio, P.; Merciaro, G.; Patrono, C.; Patrignani, P. *Circulation*, **2004,** *12*, 1468.
[74] Rinder, H.M.; Tracey, J.B.; Souhrada, M.; Wang, C.; Gagnier, R.P.; Wood, C.C. *J. Clin. Pharmacol.*, **2002,** *8*, 881.
[75] Collaborative overview of randomised trials of antiplatelet therapy--I: Prevention of death, myocardial infarction, and stroke by prolonged antiplatelet therapy in various categories of patients. Antiplatelet Trialists' Collaboration. *BMJ*, **1994,** *6921*, 81.
[76] Knijff-Dutmer, E.A.; Schut, G.A.; van de Laar, M.A. *Ann. Pharmacother.*, **2003,** *1*, 12.
[77] Schulman, S.; Henriksson, K. *Br. J. Rheumatol.*, **1989,** *1*, 46.
[78] Catella-Lawson, F.; Reilly, M.P.; Kapoor, S.C.; Cucchiara, A.J.; DeMarco, S.; Tournier, B.; Vyas, S.N.; FitzGerald, G.A. *N. Engl. J. Med.*, **2001,** *25*, 1809.
[79] MacDonald, T.M.; Wei, L. *Lancet*, **2003,** *9357*, 573.
[80] Kimmel, S.E.; Berlin, J.A.; Reilly, M.; Jaskowiak, J.; Kishel, L.; Chittams, J.; Strom, B.L. *J. Am. Coll. Cardiol.*, **2004,** *6*, 985.
[81] Mamdani, M.; Rochon, P.; Juurlink, D.N.; Anderson, G.M.; Kopp, A.; Naglie, G.; Austin, P.C.; Laupacis, A. *Arch. Intern. Med.*, **2003,** *4*, 481.
[82] Ray, W.A.; Stein, C.M.; Hall, K.; Daugherty, J.R.; Griffin, M.R. *Lancet*, **2002,** *9301*, 118.
[83] Graham, D.J.; Campen, D.; Hui, R.; Spence, M.; Cheetham, C.; Levy, G.; Shoor, S.; Ray, W.A. *Lancet*, **2005,** *9458*, 475.
[84] Hippisley-Cox, J.; Coupland, C. *BMJ*, **2005,** *7504*, 1366.
[85] Fischer, L.M.; Schlienger, R.G.; Matter, C.M.; Jick, H.; Meier, C.R. *Pharmacotherapy*, **2005,** *4*, 503.
[86] Schlienger, R.G.; Jick, H.; Meier, C.R. *Br. J. Clin. Pharmacol.*, **2002,** *3*, 327.

Received: May 15, 2006 **Revised: August 07, 2006** **Accepted: August 08, 2006**