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INVITED REVIEW

Fifty years with aspirin and platelets

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In 2021, we reached the 50th anniversary of the publication of Sir John Vane's seminal paper in *Nature New Biology* describing the experiments supporting his mechanistic hypothesis that inhibition of prostaglandin synthesis might explain the main pharmacological effects of aspirin and aspirin-like drugs, that is, reduction in pain, fever and inflammation. Bengt Samuelsson's subsequent discoveries elucidating the cyclooxygenase pathway of platelet arachidonic acid metabolism motivated my research interest towards measuring platelet thromboxane A_2 biosynthesis as a tool to investigate the clinical pharmacology of cyclooxygenase inhibition by aspirin in health and disease. What followed was a long, winding road of clinical research leading to the characterization of low-dose aspirin as a life-saving antiplatelet drug that still represents the cornerstone of antithrombotic therapy. Having witnessed and participated in these 50 years of aspirin research, I thought of providing a personal testimony of how things developed and eventually led to a remarkable success story of independent research.

KEYWORDS
aspirin, cardiac pharmacology, clinical pharmacology, cyclo-oxygenase, pharmacodynamics, platelets/thrombocytes, prostaglandins

1 | INTRODUCTION

The Second Gaddum International Lecture I had the privilege to deliver to the British Pharmacological Society (BPS) in 2021 is ideally linked in time and content with the Second Gaddum Memorial Lecture given by John Vane to the BPS in 1968 (Vane, 1969). The work described in that lecture was the direct precursor of Vane's seminal paper in *Nature New Biology* describing the experiments supporting his mechanistic hypothesis that inhibition of prostaglandin (PG) synthesis might explain the main pharmacological effects of aspirin and aspirin-like drugs, that is, reduction in pain, fever and inflammation (Vane, 1971). This Gaddum International Lecture also marks the 50th anniversary of Vane's *Nature* paper and the start of my involvement with aspirin.

In 1971, I was in New York in the laboratory of Drs Solomon Berson and Rosalyn Yalow (1977 Nobel Laureate for her discovery of the radioimmunoassay [RIA]) actively working on a project aimed at producing an antibody against $PGF_{2\alpha}$ to develop an RIA to measure this PG in human biological fluids. I had the privilege of meeting Professor Ulf von Euler (Stockholm, 1905–1983), 1970 Nobel Laureate and the 'Father' of Prostaglandins, who was in New York for a Prostaglandin Symposium and visiting with Professor Berson at Mount Sinai.

Abbreviations: COX, cyclooxygenase; CRC, colorectal cancer; ET, essential thrombocythemia; GC/MS, gas chromatography/mass spectrometry; HHT, 12L-hydroxy-5,8,10-heptadecatrienoic acid; IPD, individual participant data; MI, myocardial infarction; NSAIDs, nonsteroidal anti-inflammatory drugs; PHD, 8-(1-hydroxy-3-oxo-propyl)-9,12L-dihydroxy-5,10-heptadecadienoicacid; RCS, rabbit aorta contracting substance; RIA, radioimmunoassay; TIA, transient ischaemic attack.

This review article is based on the 2021 Gaddum International Lecture that I delivered on 1 December 2021.

Talking to him reinforced my belief that I was chasing a family of important mediators for human health and disease and that RIA had the required sensitivity and specificity to try and measure their low amounts released in vivo. Samuelsson's subsequent discoveries elucidating the cyclooxygenase (COX) pathway of platelet arachidonic acid metabolism redirected my research interest towards measuring platelet thromboxane (TX)A_2 biosynthesis as a tool to investigate the clinical pharmacology of TXA_2 inhibition by aspirin (Patrono et al., 1980). What followed was a long, winding road of clinical research leading to the characterization of low-dose aspirin as a life-saving antiplatelet drug that still represents the cornerstone of antithrombotic therapy (reviewed by Patrono, 1994, and Patrono et al., 2017).

Having witnessed and participated in these 50 years of aspirin research, I thought of providing a personal testimony of how things developed and eventually led to a remarkable success story of independent research. This article aims to tell this story, by reviewing the fundamental discoveries made by Basic Scientists and Clinical Investigators. This review is also aimed at illustrating a unique paradigm of independent drug development, based on (i) mechanistic understanding of how aspirin works in inhibiting platelet function, (ii) developing mechanism-based biomarkers to characterize the clinical pharmacology of platelet inhibition and (iii) designing adequately sized clinical trials to test realistic hypotheses of cardioprotection.

2 | BEFORE MECHANISTIC UNDERSTANDING: GUSTAV BORN AND THE PLATELET AGGREGOMETER

In one of the earliest 23,000 papers on 'aspirin AND platelets' recorded in PubMed since 1955, Louis Gast, a rheumatologist at the University of Leiden, quotes a group of French haematologists led by JL Beaumont as being the first—in 1955—to describe a prolongation of the bleeding time caused by aspirin in adult patients with cardiac disease (Gast, 1964). The paper by Gast was aimed at clarifying the pathogenesis of aspirin-induced occult faecal blood loss as well as various forms of manifest bleeding after surgical procedures. The author measured the bleeding time in three groups of patients with rheumatoid arthritis, including 57 off aspirin and 29 taking aspirin 3 g daily, and found a highly significant difference (161 vs. 302 s) in the off versus on aspirin groups, respectively (Gast, 1964). Platelet 'stickiness' was also significantly reduced by aspirin treatment, and the author suggested that 'a relation between bleeding time and platelet-stickiness seems plausible' (Gast, 1964). Interestingly, he went on to suggest that aspirin-induced faecal blood loss is caused not only by gastric or intestinal mucosal damage but also by the concomitant mild haemorrhagic diathesis, a haemostatic defect similar to that caused by some haematological disorders and anticoagulant therapy. Quite prophetically, he concluded that 'enteric-coated aspirin tablets, which presumably do not irritate the gastric mucosa, may still induce a mild haemorrhagic diathesis with subsequent increased faecal blood loss' (Gast, 1964).

So, by mid-1960s, rheumatologists using high doses (30- to 40-fold higher than currently used for cardiovascular prevention) of aspirin to treat arthritic conditions recognized bleeding complications as a side effect of aspirin administration, possibly related to impaired platelet function. However, there was no hint that the anti-inflammatory effect and the bleeding complications might reflect a common mechanism of action, something we had to wait another 10 years to realize.

In addition to playing a vital physiological role in primary haemostasis, blood platelets may aggregate intravascularly as arterial thrombi in response to fissuring or rupturing of atherosclerotic plaques, so-called 'atherothrombosis' (Davì & Patrono, 2007). Although the intravascular aggregation of platelets was described at the time of their discovery by the Italian pathologist Giulio Bizzozero in 1882, elucidation of the phenomenon and its pharmacological inhibition made little progress until it could be investigated ex vivo by the technique of optical aggregometry (Born, 1962a, 1962b; Born & Cross, 1963). The idea came to Gustav (Victor Rudolph) Born after making turbidimetric measurements of ribonuclease activity in *Streptomyces* culture filtrates carried out for the Oxford DPhil degree (reviewed by Born & Patrono, 2006). Not surprisingly, given his familial roots and cultural environment, the principle of optical platelet aggregometry developed by Gustav Born was quite simple and based on physics: As platelets aggregate in plasma in response to an agonist, such as ADP, light transmission through a cuvette containing a sample of platelet-rich plasma increases, and these time-dependent changes can be easily recorded (Born, 1962a). The last paragraph of the 1962 *Nature* paper reads: 'If it can be shown that ADP takes part in the aggregation of platelets in blood vessels, it is conceivable that AMP or some other substance could be used to inhibit or reverse platelet aggregation in thrombosis' (Born, 1962a).

In fact, optical aggregometry led to the discovery of the first platelet inhibitors, that is, ATP and adenosine, considered at first because of their close chemical relationship to pro-aggregatory ADP (Born & Cross, 1962). A few years later, these and other aggregation inhibitors were shown to be also effective in vivo, by stopping the formation and embolization of platelet thrombi in injured arterioles and venules (Born et al., 1964). Inherent limitations of this methodological approach to measuring drug-induced platelet inhibition are represented by (i) the dependence of the measured optical signal on the specific agonist (e.g., ADP) used to trigger platelet aggregation and (ii) the contribution of platelet-derived products (e.g., TXA_2), released as a consequence of platelet aggregation, to the overall response signal. As a result, aspirin was labelled as a 'weak' antiplatelet agent because its inhibitory effect on ADP-induced platelet aggregation was relatively modest and inconsistent over time, reflecting the lack of interference with the primary ADP-$P2Y_{12}$ receptor (a major platelet receptor for ADP) interaction and inhibition of the secondary response to platelet-released TXA_2 (Born & Patrono, 2006). Discovery of TXA_2 as the major arachidonic acid derivative in human platelets and a potent platelet agonist (Hamberg et al., 1975) eventually led to the utilization of arachidonic acid as a stimulus of platelet aggregation and to the appreciation that aspirin is a potent and highly effective inhibitor of this important pathway of platelet activation (reviewed by Davì & Patrono, 2007; see also Santilli et al., 2009, for a comparative assessment of different functional and biochemical assays of platelet function, as depicted in Figure 1).

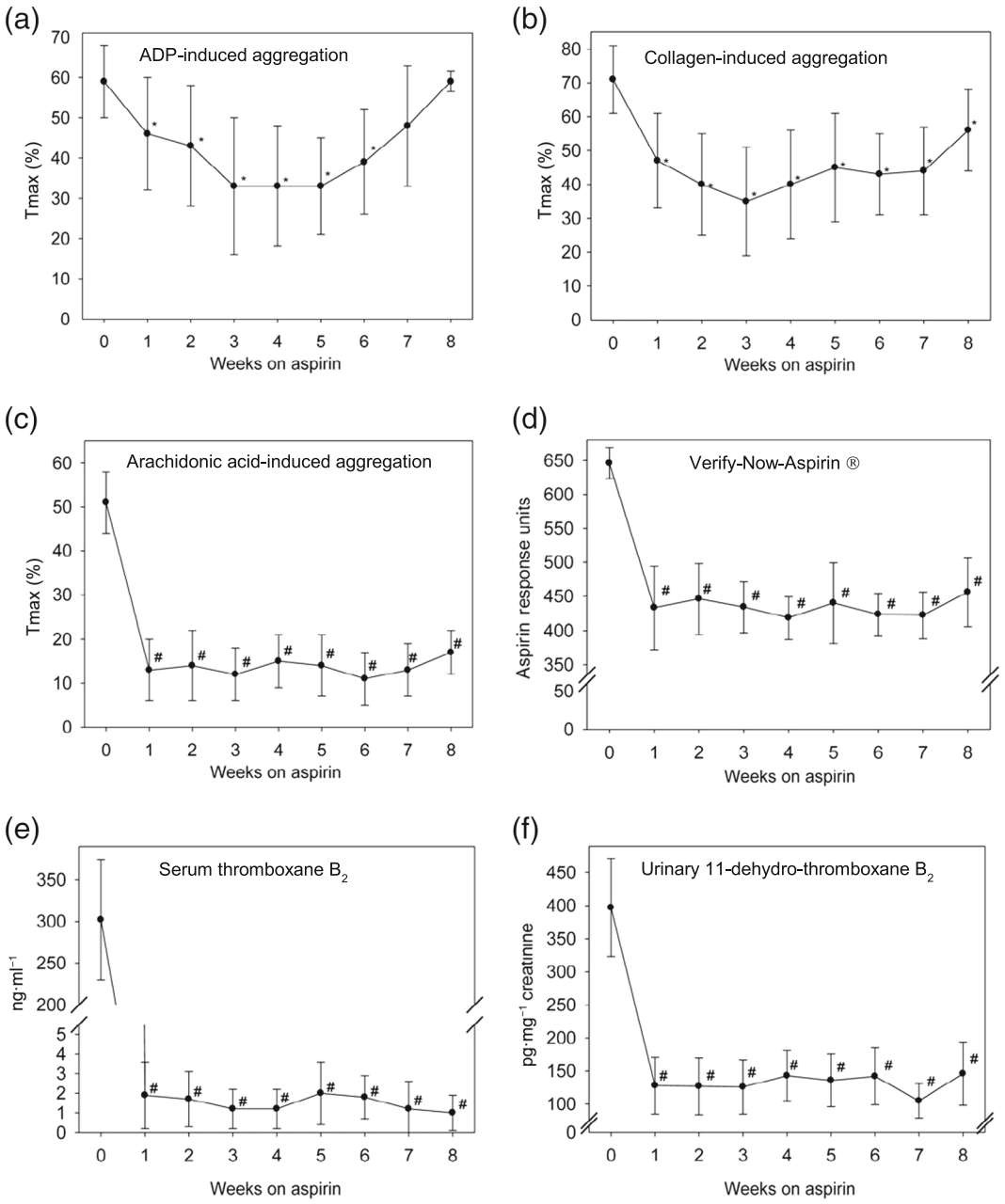

FIGURE 1 Assays of platelet biochemistry and function before and during aspirin intake in healthy volunteers. Forty-eight subjects were randomized to one of eight groups, according to treatment duration, ranging from 1 to 8 weeks. Each participant received enteric-coated aspirin 100 mg once daily. Maximal aggregation (Tmax) values of (a) ADP-, (b) collagen- and (c) arachidonic acid-induced aggregation; (d) aspirin response units of Verify-Now-Aspirin®; and absolute values of (e) serum thromboxane B_2 and (f) urinary 11-dehydro-thromboxane B_2 at baseline and during aspirin intake. Data shown are means ± SD; values were determined at baseline (Week 0, n = 48), Week 1 (n = 47), Week 2 (n = 42), Week 3 (n = 34), Week 4 (n = 28), Week 5 (n = 23), Week 6 (n = 17), Week 7 (n = 11) and Week 8 (n = 6). *$P < 0.01$, significantly different from baseline. #$P < 0.001$, significantly different from baseline. Reproduced from Santilli et al. (2009).

3 | THREE ILLUMINATING PAPERS ON THE MECHANISM OF ACTION OF ASPIRIN: THE FUNDAMENTAL DISCOVERY OF JOHN R. VANE

The first reports on the potential mechanism(s) of the antithrombotic effect of aspirin were published in 1968 by four independent groups (Evans et al., 1968; O'Brien, 1968; Weiss et al., 1968; Zucker & Peterson, 1968). The paper by Mustard's group showed that the administration of aspirin to rabbits, in doses that inhibited collagen-induced platelet aggregation, impaired haemostasis, prolonged platelet survival and diminished the amount of deposit formed in an extracorporeal shunt (Evans et al., 1968). As aspirin did not inhibit ADP-induced platelet aggregation, the same authors suggested that its

effect on the action of other stimuli was due to a decrease in the amount of ADP released (Evans et al., 1968). In the seminal paper by Weiss et al. (1968), administration of 1500 mg of aspirin, but not of sodium salicylate, produced a significant prolongation of the bleeding time in six healthy subjects when compared with the effects of placebo, an observation made earlier by Armand J. Quick who described the 'aspirin tolerance test' (1966). Moreover, aspirin ingestion resulted in reduced platelet aggregation (measured by Born's turbidimetric method) induced by (human subcutaneous) connective tissue and was associated with a decreased release of platelet ADP, whereas sodium salicylate had no effect (Weiss et al., 1968). In vitro, incubation of platelet-rich plasma with a relatively high aspirin concentration (0.5 $mM{\cdot}L^{-1}$) inhibited both the adhesion of platelets to connective tissue and the release of ADP as well as the secondary wave of platelet aggregation induced by ADP or adrenaline, whereas sodium salicylate had no effect on these reactions (Weiss et al., 1968). The inhibitory effect produced by ingesting a single 1800-mg dose of aspirin was detectable for 4–7 days, at which time salicylate was no longer detectable systemically, which suggested that aspirin produced an irreversible effect on human platelets (Weiss et al., 1968). Aspirin also inhibited the release of platelet ATP but had no effect on the platelet surface charge, available platelet ATP or ADP, or the destruction of ADP by plasma ADPase (Weiss et al., 1968). These results were interpreted by the authors as supporting the hypothesis that aspirin prolongs the bleeding time by inhibiting the release of platelet ADP, perhaps reflecting a more general inhibitory effect of this drug on membrane permeability (Weiss et al., 1968). Similar findings were reported almost simultaneously by O'Brien (1968). It is interesting to read the concluding paragraph of Harvey Weiss' paper in the *Journal of Clinical Investigation*: 'Finally, the ability of drugs such as aspirin to interfere with the hemostatic properties of platelets suggests that they may also inhibit the formation of platelet thrombi, a primary event in the formation of an arterial thrombus' (Weiss et al., 1968).

Three illuminating papers published in the same issue of *Nature New Biology* in 1971 suggested that the way in which aspirin (and aspirin-like drugs, such as indomethacin) produces its traditional pharmacological effects (reduced pain, fever and inflammation) is through inhibition of PG synthesis (Ferreira et al., 1971; Smith & Willis, 1971; Vane, 1971). However, the same mechanism—first proposed by Sir John R. Vane (1927–2004, Nobel Laureate in Physiology or Medicine in 1982)—could not explain the antiplatelet effect of aspirin because neither PGE_2 nor $PGF_{2\alpha}$, the only PGs known at the time, had any noticeable effect on platelet function.

An explanation of the mechanism of action of aspirin and other nonsteroidal anti-inflammatory drugs (NSAIDs) had long been sought in terms of inhibition of a specific enzyme or biological function. Although NSAIDs inhibited a wide variety of enzymatic reactions in vitro, no convincing relationship could be established between such inhibition and their known anti-inflammatory, antipyretic and analgesic actions. This was largely because of the high concentrations needed for enzyme inhibition (typically, in the millimolar range). In 1970, Professor John R. Vane was working in the Pharmacology Department (chaired by Professor Gustav Born) at the Royal College of Surgeons of England. He and his group of brilliant scientists (Drs Priscilla Piper, Sergio Ferreira and Salvador Moncada) had a major interest in the release and fate of vasoactive hormones and were pursuing this with special reference to the lungs. Using an original bioassay technique developed by Vane (1964), the cascade superfusion bioassay, Piper and Vane (1969) had discovered a 'rabbit aorta contracting substance' (RCS) as an unstable substance released from lungs during anaphylaxis and demonstrated that its release was inhibited by aspirin and other aspirin-like drugs. They had become interested in PGs and developed the idea that any tissue that was distorted or disturbed or traumatized would release large amounts of PGs (Piper & Vane, 1969).

At the time when Vane and his colleagues discovered that aspirin-like drugs inhibited PG biosynthesis in low concentrations (i.e., in the micromolar range), compatible with therapeutic plasma levels of NSAIDs, there was some evidence that PGs participated in the pathogenesis of inflammation and fever, and this reinforced the suggestion that inhibition of PG biosynthesis could explain the anti-inflammatory and antipyretic actions of these drugs (Vane, 1971). In fact, low concentrations of PGE_2 caused erythema and enhanced the oedema and pain induced by other inflammatory mediators, such as bradykinin. In the same seminal paper, Vane (1971) also suggested that inhibition of a PG-mediated protective mechanism against gastrointestinal mucosal damage caused by aspirin-like drugs could explain the gastrointestinal toxicity of these drugs. It is interesting to note that the word 'platelet' did not appear in his article, although an accompanying paper by JB Smith and AL Willis, graduate students of John Vane and Gustav Born in the same department, described inhibition of platelet PG synthesis by aspirin both in vitro and ex vivo, following the oral administration of 600 mg to three healthy volunteers (Smith & Willis, 1971). Similar effects were obtained with indomethacin (Smith & Willis, 1971). It is interesting to note that the use of a 600-mg dose reflected the authors' assumption that the effect of aspirin on platelet PG synthesis would require the same dose used to treat fever or pain. The conclusion of their paper reads: 'If the prostaglandins are indeed important mediators of inflammation, the clinical effectiveness of aspirin and indomethacin as anti-inflammatory agents could be explained by the inhibition of the production of prostaglandins' (Smith & Willis, 1971).

In fact, as indicated by John Vane in his autobiographical notes on the occasion of the 1982 Nobel Prize, 'From 1961 to 1973, Professor G.V.R. Born, a close friend from my Oxford days, was the Chairman of the Department of Pharmacology (at the Institute of Basic Medical Sciences of the University of London in the Royal College of Surgeons of England) and we enjoyed a strong symbiotic relationship, each maintaining an active group of graduate students and research workers. Interestingly, our fields of research endeavour (platelets and prostaglandins) only coalesced in a significant way after we had both moved on.' The way in which platelets and prostanoids coalesced in a significant way, a few years later, was through the fundamental discoveries of Bengt Samuelsson and his colleagues, who characterized the biosynthetic

intermediates in PG synthesis, that is, PGG_2 and PGH_2, and discovered a novel prostanoid with pro-aggregating activity, TXA_2, as the main derivative of PGH_2 in human platelets (Hamberg et al., 1975).

4 | THE ELUCIDATION OF THE INITIAL STEPS OF PROSTANOID BIOSYNTHESIS FROM ARACHIDONIC ACID, AND THE DISCOVERY OF TXA_2: THE FUNDAMENTAL CONTRIBUTIONS OF BENGT SAMUELSSON

So, by the early 1970s, a relatively small scientific community of people interested in haemostasis had realized that aspirin inhibited platelet function. However, a largely excessive dose of aspirin had been used to characterize its antiplatelet effects, reflecting the fact that no substantial progress had been made in understanding its mechanism of action. A major step forward in the elucidation of how aspirin works in inhibiting platelet aggregation came from the fundamental work of Bengt Samuelsson in the Chemistry Department of Karolinska Institutet in Stockholm. Professor Samuelsson shared the 1982 Nobel Prize in Physiology or Medicine with John R. Vane and Sune K. Bergström (Stockholm, 1916–2004). Interestingly, the discovery of evanescent, biologically active compounds by Vane, a chemist turned pharmacologist, provided a stimulus for the chemical trapping and structural characterization of these compounds as novel prostanoids by Samuelsson, a medical doctor turned chemist.

The early work of Mats Hamberg and Bengt Samuelsson (1973) demonstrated that the biosynthesis of PGs occurs in a stepwise fashion with several enzymes involved and not by a concerted reaction with a single enzyme. They identified an endoperoxide intermediate in PG biosynthesis and proposed the existence of an endoperoxide isomerase that catalyses rearrangement of the endoperoxide intermediate into PGE compounds and an endoperoxide reductase that catalyses reduction of the endoperoxide into PGF compounds (Hamberg & Samuelsson, 1973). Moreover, their work demonstrated that the endoperoxide intermediate had strong stimulating activity on the rabbit aorta strip and suggested that it might account, at least in part, for the biological activity of the indomethacin-suppressable RCS from guinea pig lung first described by Piper and Vane (1969). Subsequent work by Hamberg, Svensson, Wakabayashi and Samuelsson (1974) led to the isolation and structure elucidation of two PG endoperoxides, named PGG_2 and PGH_2, that caused platelet aggregation. This discovery made it likely that PGG_2 is the first stable compound formed from arachidonic acid by the enzyme known at the time as 'prostaglandin synthetase' (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974). Through the isolation of PGG_2, it was demonstrated for the first time that introduction of the oxygen function at C-15 of the PG structure occurs by a dioxygenase reaction. Hamberg, Svensson, Wakabayashi and Samuelsson (1974) proposed the name 'fatty acid cyclo-oxygenase' for the enzyme that catalyses the conversion of arachidonic acid into PGG_2 by oxygenation at C-11 and C-15. Furthermore, their findings that PGG_2 and/or PGH_2 were formed during thrombin-induced platelet aggregation and that they were powerful aggregating agents suggested that these unstable PG intermediates played a role in platelet aggregation (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974). The last paragraph of this seminal paper reads: 'Aspirin, an inhibitor of prostaglandin biosynthesis in platelets, has been reported to inhibit the second phase of platelet aggregation. It is suggested that this effect is due to inhibited formation of PGG_2 and PGH_2 from arachidonic acid within the platelet' (Hamberg, Svensson, Wakabayashi & Samuelsson, 1974).

Additional work from Samuelsson's group led to a new concept whereby PGs can exert their biological action through the endoperoxides, and these compounds may be metabolized almost exclusively to non-prostanoate structures and only to a smaller extent to the classical PGs (Hamberg, Svensson & Samuelsson, 1974). The hemiacetal derivative of 8-(1-hydroxy-3-oxo-propyl)-9,12L-dihydroxy-5,10-heptadecadienoicacid (PHD) and 12L-hydroxy-5,-8,10-heptadecatrienoic acid (HHT) were found to be the major metabolites of PGG_2 in suspensions of human platelets (Hamberg, Svensson & Samuelsson, 1974). Conversion of PGG_2 into PHD was suggested to occur by rearrangement of the endoperoxide structure followed by incorporation of one molecule of H_2O. Hamberg et al. (1975) went on to demonstrate the formation of an unstable, biologically active oxane intermediate between PGG_2 and PHD in human platelets. The name 'thromboxanes' was introduced to designate this new group of compounds of which the unstable intermediate (half-life, 34 s) is TXA_2 and the previously recognized compound, provisionally called PHD, is TXB_2 (Hamberg et al., 1975). These exciting findings were first communicated by Bengt Samuelsson in May 1975 at the International Prostaglandin Conference in Florence. Incubation of arachidonic acid or PGG_2 with washed platelets led to formation of an unstable factor that induced irreversible platelet aggregation and caused the release of [^{14}C] 5-HT from platelets that had been incubated with [^{14}C] 5-HT. The properties and the mode of formation of this factor indicated that it was identical with TXA_2. Furthermore, evidence was presented that the more unstable and major component of RCS formed in platelets and guinea pig lung is also TXA_2 (Hamberg et al., 1975).

As a personal note, by the time I attended the 1975 Prostaglandin Conference and listened to Samuelsson's presentation on the discovery of TXA_2, our group of young clinical pharmacologists at the Catholic University School of Medicine in Rome had started investigating the effects of aspirin and other NSAIDs on PG production in surgical samples of human synovial tissue and intact platelets perfused with PG inhibitors in vitro (Patrono et al., 1976). We presented evidence at the same conference that platelets were sixfold more sensitive to aspirin inhibition than synovium, whereas these model systems were equally sensitive to indomethacin and fenoprofen (Patrono et al., 1976), an observation that—together with the discovery of TXA_2—triggered our interest in investigating the clinical pharmacology of aspirin as an antiplatelet agent (see below).

5 | THE IRREVERSIBLE NATURE OF PGG/H-SYNTHASE INACTIVATION BY ASPIRIN THROUGH SELECTIVE ACETYLATION OF A CRITICAL SERINE RESIDUE: THE WORK OF PHILIP MAJERUS

By the time Samuelsson had discovered TXA_2 and characterized its biosynthetic route in human platelets (Hamberg et al., 1975), aspirin had been shown to acetylate proteins such as albumin, immunoglobulins and fibrinogen (Hawkins et al., 1969; Pinckard et al., 1968). However, these acetylation processes occurred slowly over hours and required high concentrations in the 1–20 mM range. In contrast, aspirin inhibited COX under clearly different conditions, namely, within minutes at concentrations in the micromolar range (Hamberg & Samuelsson, 1974). By using suspensions of washed human platelets incubated at 37°C with [^{3}H] aspirin, Gerald Roth and Philip Majerus (1936–2016), physicians/scientists working in the Hematology-Oncology Division of Washington University in St. Louis, characterized the mechanism responsible for permanent inactivation of COX activity by aspirin (Roth et al., 1975; Roth & Majerus, 1975). Exposure to [*acetyl*-^{3}H] aspirin but not to [*aromatic ring*-^{3}H] aspirin resulted in radioactive labelling of three platelet proteins (Roth & Majerus, 1975). The acetylation of two of the proteins located in the supernatant fraction was not saturable. Acetylation of the third protein, approximate molecular weight 85,000 located in the particulate fraction, saturated at an aspirin concentration of 30 μM and was complete within 20 min (Roth & Majerus, 1975). Platelets prepared from aspirin-treated donors did not incorporate any [*acetyl*-^{3}H] aspirin radioactivity into the particulate protein for 2 days after drug treatment, indicating that acetylation of the protein was completely inhibited, and did not show full pretreatment uptake of radioactivity for 12 days thereafter. The time course of progressively increasing incorporation of [*acetyl*-^{3}H] aspirin radioactivity paralleled that of platelet turnover, substantiating the idea that acetylation of this protein by aspirin was a permanent effect (Roth & Majerus, 1975). Based on these findings, Roth and Majerus (1975) suggested that aspirin may exert its antiplatelet effect by acetylating a residue within an active site of the PGG/H-synthase. The same investigators reported aspirin-mediated acetylation of a single microsomal protein (molecular weight 85,000) in sheep and bovine seminal vesicles and human platelets and provided experimental evidence that aspirin inhibits PG-synthase activity in these tissues by acetylating the enzyme (Roth et al., 1975). They concluded that this action of aspirin may account for its anti-inflammatory and antiplatelet actions (Roth et al., 1975) (Figure 2).

Further clinical work by Majerus' group exploited the ability of ingested aspirin to inhibit subsequent in vitro acetylation of COX in washed platelets by [^{3}H-acetyl] aspirin, to investigate the sensitivity of human platelets to aspirin (Burch, Baenziger, et al., 1978). The underlying assumption of these studies was that the measured inhibition of in vitro COX acetylation in washed platelets by [^{3}H-acetyl] aspirin reflects the degree of unmeasured platelet PG synthesis inhibition (Burch, Baenziger, et al., 1978). Tritium incorporation into platelet COX was measured in serial blood samples obtained from 16 healthy subjects who were given a single 325-mg aspirin dose, as depicted in Figure 3a. An initial fall in tritium incorporation to 11% of predosing values was detected 6 h after aspirin ingestion, with no apparent change in the degree of COX acetylation during the following 2 days (Figure 3a). Thereafter, the time course of COX acetylation suggested that unacetylated enzyme returned to the circulation in a time-dependent fashion consistent with platelet turnover (calculated platelet life span, 8.2 ± 2 days) (Burch, Baenziger, et al., 1978). This 2-day 'lag' was interpreted as indirect evidence that aspirin also acetylates COX in the bone-marrow megakaryocytes (Burch, Baenziger, et al., 1978). In the same study, four groups of healthy subjects were given single aspirin doses of 20, 80, 160 and 650 mg, and the degree of platelet COX acetylation was measured before and 24 h after oral dosing. Burch, Baenziger, et al. (1978) found a direct relationship between the amount of aspirin ingested and the degree of enzyme inactivation. Daily doses of 20–325 mg given for 5 to 7 days produced 61% to >95% COX acetylation 24 h after the last aspirin ingestion (Figure 3b). Burch, Baenziger, et al. (1978) concluded that 'It is even conceivable that doses as low as 20 mg per day might have antithrombotic action inasmuch as the degree to which cyclooxygenase must be inhibited to alter thrombosis is unknown.'

Based on additional in vitro studies, Majerus' group also reported that at least 10 times as much aspirin would be required to inhibit

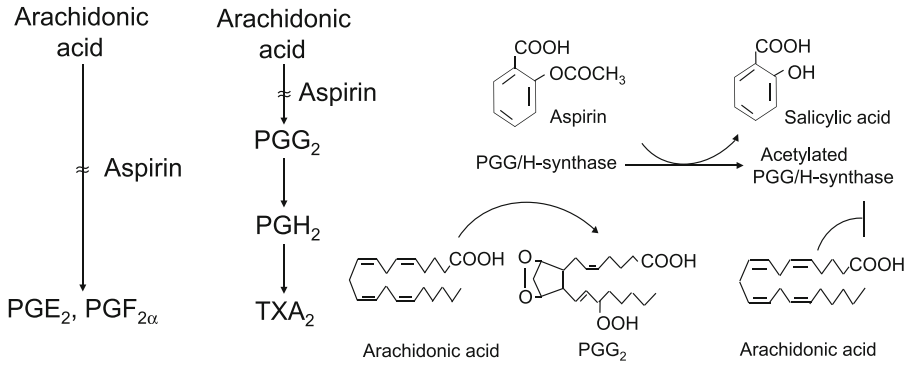

FIGURE 2 The three main steps in the understanding of the molecular mechanism of action of aspirin in inhibiting thromboxane-dependent platelet function. See text for details of these discoveries.

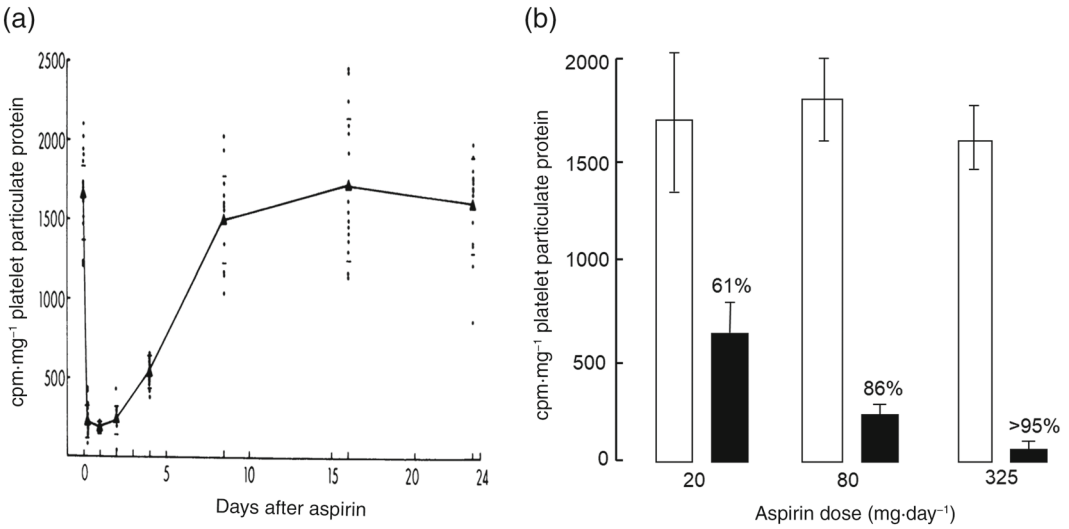

FIGURE 3 Clinical pharmacology of platelet cyclooxygenase acetylation. Panel (a) depicts reappearance of active (unacetylated) cyclooxygenase in the circulation after a single 325-mg aspirin dose. Values are presented as tritium incorporation (counts per minute) per milligram platelet particulate protein. Protein recovery did not vary significantly at the different time points. Data shown are individual values with means ± SD. Panel (b) depicts the effect of daily aspirin on platelet cyclooxygenase. Data shown are means ± SD. Open bars represent mean cyclooxygenase level before aspirin ingestion. Solid bars represent mean cyclooxygenase level 24 h after cessation of drug. Percentage figures above each solid bar indicate the percent inhibition attained by the respective aspirin dose. Number of subjects participating in the experiment was 6, 8 and 6 for the 20-, 80- and 325-mg doses, respectively. Reproduced from Burch, Stanford and Majerus (1978).

vascular COX as is required to inactivate platelet COX (Burch, Stanford & Majerus, 1978), consistent with our earlier finding of differential inhibition of PG synthesis in human platelets versus synovial tissue (Patrono et al., 1976).

In order to provide proof of concept of the potential antithrombotic effect of relatively low doses of aspirin, Majerus' group went on to test the efficacy of aspirin, 160 mg daily, in preventing shunt thrombosis in 44 patients undergoing chronic haemodialysis, in a randomized, double-blind, placebo-controlled trial (Harter et al., 1979). Thrombi occurred in 72% of the patients on placebo and 32% of those treated with aspirin ($P < 0.01$). However, as outlined by the authors' concluding remarks, 'The finding that aspirin prevents shunt thrombosis does not indicate that it prevents death from myocardial infarction' (Harter et al., 1979). We had to wait another decade of aspirin research before the Second International Study of Infarct Survival (ISIS-2) clearly demonstrated that the same aspirin dose used by Harter et al. (1979) in 44 haemodialysed patients actually reduced vascular mortality in over 17,000 patients with a suspected acute myocardial infarction (MI) (ISIS-2 Collaborative Group, 1988).

6 | THE CLINICAL PHARMACOLOGY OF PLATELET TX INHIBITION BY ASPIRIN: THE WORK OF CARLO PATRONO AND GARRET FITZGERALD

Mechanistic understanding of the way in which aspirin inhibits platelet function provided a rationale for revisiting the way in which we assessed its antiplatelet pharmacodynamics (reviewed by Born & Patrono, 2006). Born's aggregometer measured a non-specific electrical signal in response to a specific stimulus, such as ADP. This approach lacked adequate specificity and sensitivity to assess the effects of aspirin on the COX pathway of platelet arachidonic acid metabolism. Moreover, based on platelet aggregation measurements following aspirin administration, it was thought that daily doses in the range of 1000–1500 mg would be required in order to obtain maximal effects on coronary atherothrombosis (AMIS Research Group, 1980; Breddin et al., 1980). The German-Austrian aspirin trial and the Aspirin Myocardial Infarction Study (AMIS) tested this hypothesis and reported non-significant, conflicting changes in total mortality, that is, 18% decrease (based on 59 deaths) and 11% increase (based on 463 deaths), respectively (AMIS Research Group, 1980; Breddin et al., 1980).

Publication of these disappointing results coincided with publication of two papers describing novel methodological approaches to measurement of TXA_2 biosynthesis in man (Patrono et al., 1980; Roberts et al., 1981). Our group developed a method for assessing the ex vivo platelet production of TXB_2 in response to endogenously formed thrombin, by allowing 1-ml whole blood samples to clot in a glass test tube at 37°C for 30 min and measuring TXB_2 concentrations by RIA in the separated serum (Patrono et al., 1980). The concentrations of generated TXB_2 averaged 222 ± 81 (SD) ng·ml^{-1} of serum from 45 healthy subjects and were highly reproducible in the same subject upon repeated sampling. A single 100-mg aspirin dose reduced serum TXB_2 by 98% during the first hour. Single doses of 100- to 400-mg aspirin resulted in 94%–98% inhibition after 24 and 48 h and 90%–92% after 72 h.

Thereafter, serum TXB_2 returned to control levels with a time course consistent with platelet turnover (Patrono et al., 1980). The advantage of this approach over measurement of platelet aggregation with Born's aggregometer was related to (i) a much higher signal-to-noise ratio, as the biochemical signal could be virtually abolished by a drug inactivating platelet COX-1 activity; (ii) inherent pharmacodynamic specificity for investigating the effects of COX-1 or TX-synthase inhibitors; and (iii) analytical sensitivity and specificity provided by RIA for detecting very low concentrations of serum TXB_2 (Patrono et al., 1980). This method was rapidly adopted by other investigators (Pedersen & FitzGerald, 1984; Weksler et al., 1983) and still represents the gold standard for investigating aspirin pharmacodynamics (Catella-Lawson et al., 2001; Li et al., 2014).

Jack Roberts at the Division of Clinical Pharmacology of Vanderbilt University in Nashville investigated the metabolic fate of systemically infused [$^3H_8$] TXB_2 into a healthy adult male and reported the formation of 20 metabolites, which were detected in urine by gas chromatography/mass spectrometry (GC/MS) (Roberts et al., 1981). The discovery of 2,3-dinor-TXB_2 as a major enzymatic metabolite of TXB_2 (TXM) paved the way for investigating TXA_2 biosynthesis in vivo and its pharmacological modulation by aspirin (FitzGerald, Oates, et al., 1983; Patrono et al., 1986). Moreover, this analytical approach provided a non-invasive biomarker for assessing transient as well as persistent changes in TXA_2-dependent platelet activation, in the acute phase of coronary and cerebrovascular ischaemic syndromes as well as in association with several cardiovascular risk factors, respectively (reviewed by Davì & Patrono, 2007).

Similar PGI_2 infusion studies in healthy subjects allowed the metabolic fate of this antiplatelet and vasodilator prostanoid to be characterized, thus demonstrating that it was unlikely to function as a circulating hormone (FitzGerald et al., 1981; Patrono et al., 1982). Furthermore, the GC/MS characterization of 2,3-dinor-6-keto-$PGF_{1\alpha}$ (PGIM) as a major urinary metabolite of systemically infused PGI_2 (FitzGerald et al., 1981) and the evidence that urinary excretion of the non-enzymatic hydrolysis product of PGI_2, 6-keto-$PGF_{1\alpha}$, reflected primarily its renal synthesis (Patrono et al., 1982) provided the analytical tools to investigate the clinical pharmacology of platelet inhibition by aspirin and to demonstrate its relative biochemical selectivity at low doses in healthy subjects (FitzGerald, Oates, et al., 1983; Patrignani et al., 1982). We reported that single aspirin doses of 6–100 mg resulted in log-linear inhibition of platelet TXB_2 production, ranging from 12% to 95% after 24 h (Figure 4a) (Patrignani et al., 1982). A daily dose of 30 mg given for 7 days produced a cumulative and virtually complete suppression of platelet TXB_2 production, without significantly reducing the urinary excretion of 6-keto-$PGF_{1\alpha}$ (Figure 4b). The platelet inhibitory effect of this low-dose aspirin regimen was maintained unaltered throughout 1 month of therapy, with no evidence of cumulative inhibition of renal PGI_2 synthesis. Moreover, furosemide-induced renal PGI_2 synthesis and renin release were unaffected by chronic low-dose aspirin. Following cessation of aspirin therapy, platelet TXB_2 production returned towards control values at a rate consistent with platelet turnover (Figure 4b) (Patrignani et al., 1982). This study demonstrated

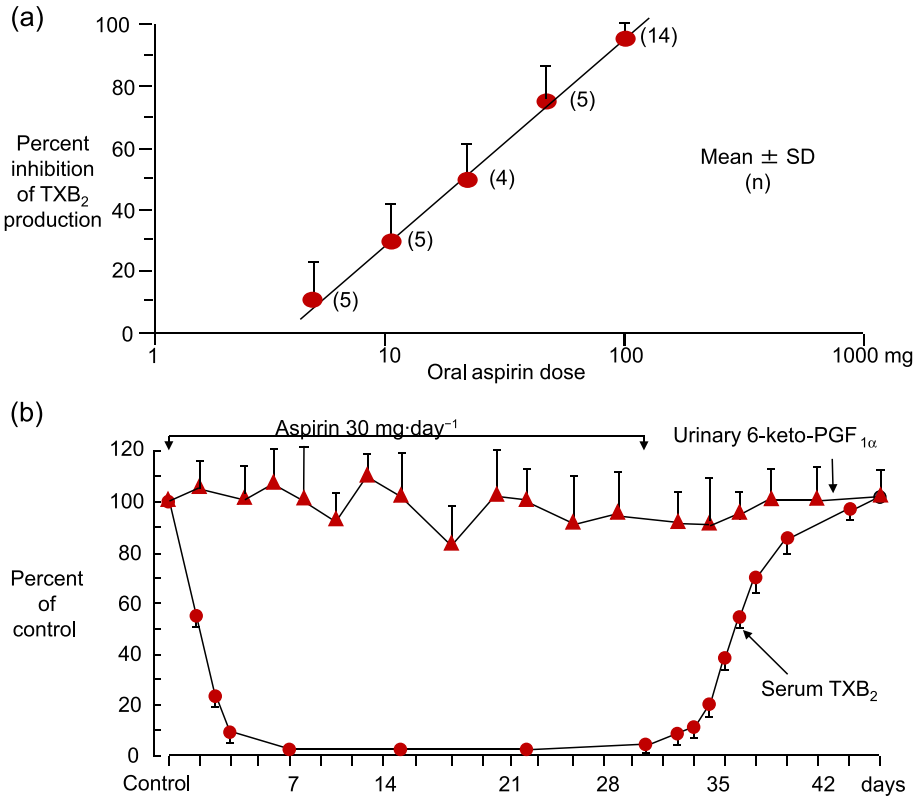

FIGURE 4 Clinical pharmacology of platelet thromboxane (TX) inhibition. Panel (a) depicts log-linear inhibition of platelet cyclooxygenase activity by aspirin in healthy subjects. TXB_2 production during whole blood clotting was measured before and 24 h after oral aspirin ingestion. The results are expressed as percent inhibition, each subject serving as his or her own control. Data shown are means ± SD. Numbers in parentheses indicate the number of subjects for each dose of aspirin. Panel (b) shows selective cumulative inhibition of platelet TXA_2 production by low-dose aspirin in healthy subjects. Serum TXB_2 concentrations and urinary excretion of 6-keto-PGF_{1a}, expressed as percentage of pre-aspirin values, were measured in three healthy subjects before, during and after aspirin 30 mg daily. Data shown are means ± SEM. The arrows indicate duration of daily aspirin intake. Redrawn from Patrignani et al. (1982).

that during chronic low-dose aspirin therapy, at a dose that was only 2% to 3% of the daily doses used in contemporary post-MI trials (AMIS Research Group, 1980; Breddin et al., 1980), renal PGI_2-producing cells were readily activated by furosemide, concomitant with virtually complete suppression of platelet TXA_2 biosynthesis (Patrignani et al., 1982). The same low-dose aspirin regimen was also shown to be effective in the long-term inhibition of TXA_2-dependent platelet function in patients with a recent MI (De Caterina et al., 1985). In additional studies, it was shown that the fractional dose of aspirin necessary to achieve a given level of serum TXB_2 inhibition by virtue of cumulative effects approximately equals the fractional daily platelet turnover (Patrono et al., 1985). Serum TXB_2 measurements obtained during long-term dosing with 0.11, 0.22 and 0.44 $mg \cdot kg^{-1}$ aspirin in four healthy subjects could be fitted by a theoretical model assuming identical acetylation of platelet (irreversible) and megakaryocyte (reversible) COX-1 (Patrono et al., 1985). For a given dose within this range, both the rate at which cumulative acetylation occurs and its maximal extent largely depend upon the rate of platelet turnover (Patrono et al., 1985).

FitzGerald, Oates, et al. (1983) performed an extended study in five healthy subjects to relate aspirin intake, over a wide range of doses, to both TXA_2 and PGI_2 biosynthesis, as reflected by urinary TXM and PGIM, respectively. Aspirin, in the range of 20 to 325 $mg \cdot day^{-1}$, resulted in a dose-dependent decline in both TXM and PGIM excretion. At doses of 325–2600 $mg \cdot day^{-1}$, TXM excretion ranged from 5% to 3% of control values whereas PGIM remained at 37% to 23% of control (FitzGerald, Oates, et al., 1983). It should be emphasized that, whereas serum TXB_2 reflects exclusively platelet TXA_2 production (Patrono et al., 1980), urinary TXM is derived from both platelet and extra-platelet sources of TXA_2 (FitzGerald, Pedersen & Patrono, 1983). This probably explains the conclusion that 'it is unlikely that any dose of aspirin can maximally inhibit thromboxane generation without also reducing endogenous prostacyclin biosynthesis' (FitzGerald, Oates, et al., 1983). Using a different approach, Weksler et al. (1983) studied the ability of single oral doses of aspirin, in the range of 40 to 325 mg, to inhibit serum TXB_2 and PGI_2 synthesis by human arterial and venous tissue obtained from 70 patients undergoing aortocoronary bypass. The results of this study provided further evidence for the biochemical selectivity of low-dose aspirin (80 mg), as it had only a limited effect on PGI_2 production in venous and arterial endothelium (19% to 38% inhibition), while reducing serum TXB_2 by 95% (Weksler et al., 1983).

Overall, these studies indicated that the effect of daily administration of aspirin on platelet TXA_2 production was saturable at low doses because of the cumulative nature of its inhibition (Patrignani et al., 1982), consistent with saturability of COX-1 acetylation (Burch, Stanford & Majerus, 1978), whereas vascular PGI_2 biosynthesis was dose-dependently reduced and required higher doses (650–1300 mg) to reach its nadir (FitzGerald, Oates, et al., 1983). It is interesting to note that in a *New England Journal of Medicine* 1983 Editorial on Aspirin as an Antithrombotic Medication, Aaron J. Marcus (1983) concluded 'At present, my colleagues and I recommend a single dose of aspirin (325 mg) daily accompanied by dipyridamole (about 75 mg) three times daily for patients at risk of occlusive arterial disease of the cerebral, coronary, or peripheral circulation by virtue of a strongly positive family history and for those with documented previous episodes', largely reflecting his view that the concept of an 'aspirin dilemma' (Bertelé et al., 1983) was no longer of clinical relevance. In response to this draconian statement, I argued that 'whether a selective inhibition of thromboxane A_2-dependent platelet function by low-dose aspirin will offer increased antithrombotic efficacy, fewer toxic reactions, or both should not represent a topic for philosophical dissertations on the importance of prostacyclin in life but rather should provide a working hypothesis for further clinical investigation' (Patrono, 1984). To put things in perspective, at around the same time, Jim Chesebro, Valentin Fuster and colleagues at the Mayo Clinic were using dipyridamole 75 mg and aspirin 325 mg, to be given three times daily, to reduce the risk of vein-graft occlusion after coronary bypass operations (Chesebro et al., 1984); similarly, John Cairns and colleagues at the McMaster University were using aspirin 325 mg four times daily to treat unstable angina (Cairns et al., 1985), based on the (erroneous) premise that aspirin had dose-dependent antithrombotic effects unrelated to COX acetylation (reviewed by Patrono et al., 1998). We had to wait a few more years for the TX/prostacyclin hypothesis (Moncada & Vane, 1979) to be tested by a series of randomized clinical trials evaluating the efficacy and safety of low-dose aspirin in the acute treatment and secondary prevention of coronary and cerebral atherothrombosis (reviewed by Patrono, 1994).

7 | THE CLINICAL EFFICACY AND SAFETY OF LOW-DOSE ASPIRIN: THE WORK OF RORY COLLINS, PETER SLEIGHT, RICHARD PETO, LARS WALLENTIN, JAN VAN GIJN, BO NORRVING AND MANY OTHERS

Between 1984 and 1989, over 22,000 patients with acute or chronic atherosclerotic vascular disease were recruited into four multicentre, randomized clinical trials testing the efficacy and safety of low-dose aspirin (30 to 162.5 mg once daily) versus placebo (ISIS-2 Collaborative Group, 1988; The RISC Group, 1990; The SALT Collaborative Group, 1991) or a higher dose (The Dutch TIA Trial Study Group, 1991). Lars Wallentin, Bo Norrving and Jan van Gijn led the teams in Sweden and the Netherlands that were responsible for the design and multicentre implementation of the very first trials of low doses of aspirin given once daily for the secondary prevention of coronary and cerebrovascular events. The results of these trials established that (i) an aspirin daily dose of 75 mg was effective in reducing the risk of fatal and non-fatal atherothrombotic complications in patients with unstable coronary artery disease (The RISC Collaborative Group, 1990) and those with a recent transient ischaemic attack (TIA) or minor ischaemic stroke (The SALT Collaborative Group, 1991) and that (ii) a daily dose as low as 30 mg was no less effective in the prevention of serious vascular events than a 283-mg dose and produced fewer adverse effects, including fewer major and minor bleeding complications, in patients with a recent TIA or minor

ischaemic stroke (The Dutch TIA Trial Study Group, 1991). The ISIS-2 trial, coordinated by Sir Rory Collins at the University of Oxford, demonstrated that a daily dose of 162.5 mg, initiated within 24 h after the onset of symptoms of a suspected acute MI and continued for 5 weeks, significantly reduced 5-week vascular mortality by about a quarter and non-fatal reinfarction and non-fatal stroke by half and was not associated with any significant increase in cerebral haemorrhage or in other major bleeds (ISIS-2 Collaborative Group, 1988). With a 2 × 2 factorial design, ISIS-2 also showed that aspirin-based antithrombotic treatment and streptokinase-based fibrinolytic therapy were equally effective in improving survival after acute MI and that their effects were additive, by reducing 5-week vascular mortality by 42%, supporting the concept of coronary atherothrombosis as a multifactorial, dynamic process (ISIS-2 Collaborative Group, 1988). It is interesting to note that in the discussion of their *Lancet* 1988 paper, the ISIS-2 investigators wrote 'The optimum dose, and frequency of dosing, of aspirin remains uncertain. If the chief mechanism is inhibition of cyclo-oxygenase-dependent platelet aggregation, then any daily dose from about 40 mg upwards may suffice.'

Indirect comparisons among aspirin trials in high-risk patients performed over the following 30 years (Antithrombotic Trialists' Collaboration, 2002), as well as a limited number of direct, randomized dose comparisons (Jones et al., 2021; The CURRENT-OASIS 7 Investigators, 2010), have largely confirmed this prediction by showing that higher doses are not more effective than lower doses and may be associated with a higher risk of bleeding complications, particularly gastrointestinal bleeds. Thus, in the largest aspirin dose comparison performed by the CURRENT-OASIS 7 Investigators (2010) in over 25,000 patients with acute coronary syndromes, there was a statistically significant increase in the incidence of major gastrointestinal bleeding among patients who received higher dose aspirin (300–325 mg daily), as compared with those who received lower dose aspirin (75–100 mg daily), with comparable efficacy in preventing death from cardiovascular causes, MI or stroke.

The finding of saturability of the antithrombotic effect of aspirin at low doses is consistent with saturability of the platelet COX-1 acetylation process (Patrignani et al., 2014) and the inhibition of platelet TXA_2 production (Patrignani et al., 1982) at similar doses (Figure 5), as discussed above, and supports the contention that this mechanism of action can fully account for the clinical efficacy of low-dose aspirin in the prevention of atherothrombosis (Patrono et al., 2005).

As for the clinical relevance of the TX/prostacyclin hypothesis (Moncada & Vane, 1979), there is no large randomized comparison of low-dose aspirin versus a sufficiently high dose (e.g., 650 to 1300 mg daily) to profoundly suppress PGI_2 biosynthesis, except for the ASA and Carotid Endarterectomy (ACE) Trial, which was actually designed to test the opposite hypothesis (Taylor et al., 1999). Approximately 3000 patients scheduled for carotid endarterectomy were randomly assigned to receive daily doses of 81-, 325-, 650- or 1300-mg aspirin, started before surgery and continued for 3 months. Contrary to the authors' expectation, the combined rate of stroke, MI or death at 3 months was significantly ($P = 0.03$) lower in the low-dose groups (6.2%) than in the high-dose groups (8.4%) (Taylor et al., 1999).

Perhaps, more convincing evidence for the pathophysiological relevance of PGI_2 as a COX-2-derived mediator of endothelial thromboresistance (McAdam et al., 1999) was provided by the widespread use of coxibs, a class of selective COX-2 inhibitors (reviewed by FitzGerald & Patrono, 2001), and the placebo-controlled

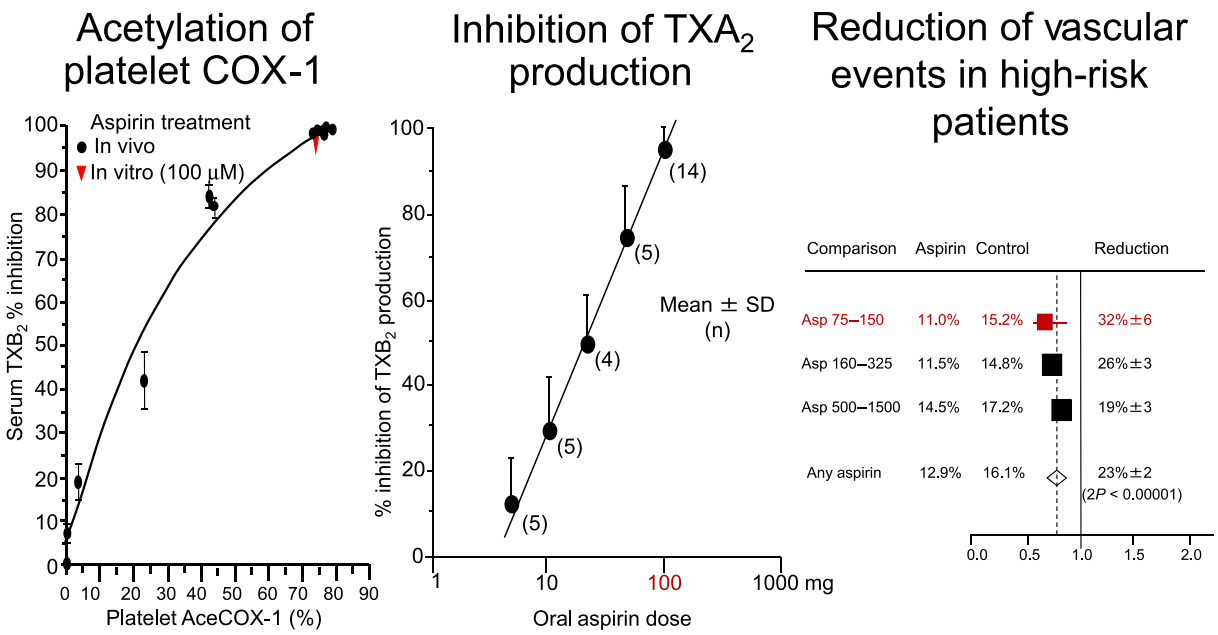

FIGURE 5 Acetylation of platelet cyclooxygenase (COX)-1, inhibition of thromboxane (TX)B_2 production and reduction of vascular events by aspirin are saturable at low doses. The left panel is redrawn from Patrignani et al. (2014); the centre panel is redrawn from Patrignani et al. (1982); and the right panel is redrawn from Antithrombotic Trialists' Collaboration (2002).

chemopreventive trials that revealed the cardiovascular hazard of rofecoxib and celecoxib (reviewed by Patrono & Baigent, 2014). The individual participant data (IPD) meta-analyses of all the published and unpublished randomized trials of any NSAID versus placebo or any NSAID versus another comparator NSAID(s) performed by the Coxib and traditional NSAID Trialists' (CNT) Collaboration established that the increased risk of atherothrombotic complications represents a mechanism-based class effect of COX-2 inhibitors, regardless of COX-isozyme selectivity (CNT Collaboration, 2013). The pattern of serious cardiovascular effects of COX-2 inhibitors (primarily, a twofold increase in MI) is consistent with the hypothesis that the enhanced risk of coronary events (largely atherothrombotic in nature) is related to an impaired restraining effect of endothelial PGI_2 on platelet activation at sites of atherosclerotic plaque rupture or fissuring (Grosser et al., 2010; Patrono, 2016).

8 | IS ONE ASPIRIN DOSING REGIMEN THE OPTIMAL CHOICE FOR ALL? ASPIRIN 'RESISTANCE' REVISITED

Low-dose aspirin can prevent about one quarter of serious vascular events in high-risk patients (Antithrombotic Trialists' Collaboration, 2002). Patients may experience recurrent events while on aspirin because of the multifactorial nature of atherothrombosis (Davì & Patrono, 2007). Such a treatment failure has been inappropriately referred to as aspirin 'resistance' (reviewed by Patrono & Rocca, 2007). This term has been used also to indicate less-than-expected inhibition of platelet aggregation by low-dose aspirin, with variable estimates of its incidence and inconclusive data on its clinical significance (Patrono & Rocca, 2007). Among the authors of >3000 publications on the topic, Eric Topol and colleagues at the Cleveland Clinic proposed that aspirin 'resistance' represents a true clinical entity, requiring a change in antiplatelet therapy when it is diagnosed (Gum et al., 2003). Intrinsic to this suggestion is the underlying assumption that a single measurement of agonist-induced platelet aggregation can determine whether aspirin has fully inhibited platelet COX activity and define a stable 'resistant' or 'non-responder' phenotype based on arbitrary thresholds of functional response (Patrono & Rocca, 2007).

Santilli et al. (2009) compared different functional and biochemical assays for their capacity to assess the antiplatelet effect of aspirin 100 mg given orally for 1 to 8 weeks to 48 healthy subjects (Figure 1). Novel aspects of the study design included up to eight repeated measurements in the same subjects to assess the intrasubject reproducibility of the various assays, as well as analysis of the recovery dynamics following aspirin withdrawal (Santilli et al., 2009). As shown in Figure 1, whereas serum TXB_2 was consistently suppressed by at least 97%, compared with baseline, in over 200 determinations, measurements of various functional indexes categorized according to previously described response thresholds identified 1.4% to 30% of 'nonresponder' samples (Santilli et al., 2009). However, prior or subsequent determinations performed in the same subjects clearly identified the fluctuating nature of this apparent 'nonresponder' phenotype, most likely reflecting the relatively poor intrasubject reproducibility of functional measurements (Santilli et al., 2009). We also suggested that the strikingly non-linear relationship between inhibition of TXA_2 production and inhibition of TXA_2-dependent platelet function (Reilly & FitzGerald, 1987) may be responsible for some recovery of platelet function during the 24-h dosing interval, a phenomenon likely to be more pronounced in some subjects because of the interindividual variability in platelet turnover (Santilli et al., 2009). Based on a study of 400 healthy subjects, Grosser et al. (2013) concluded that pharmacological resistance to aspirin is rare. In fact, this study failed to identify a single case of true drug resistance. Pseudo-resistance, reflecting delayed and reduced drug absorption, may be apparent following enteric-coated but not immediate release aspirin administration (Grosser et al., 2013).

Subsequent work by Bianca Rocca and colleagues at the Catholic University in Rome demonstrated substantial interindividual variability in the rate of recovery of platelet COX-1 activity during the 24-h dosing interval, in stable patients with atherosclerotic cardiovascular disease, requiring more frequent dosing in patients with accelerated renewal of the drug target (Rocca et al., 2012). These included about one third of the population with Type-2 diabetes mellitus (Rocca et al., 2012), patients undergoing on-pump cardiac surgery (Cavalca et al., 2017) and those with essential thrombocythemia (ET) (Dragani et al., 2010; Pascale et al., 2012).

ET is a myeloproliferative neoplasm characterized by high platelet generation and activation, and enhanced thrombotic risk (Patrono et al., 2013). As shown in Figure 6, bone-marrow megakaryocytes express both COX-1 and COX-2 (Rocca et al., 2002). Newly formed platelets, derived from fragmentation of megakaryocyte cytoplasm, also transiently express both COX-isoforms, but mature blood platelets only express COX-1 (Rocca et al., 2002). Pascale et al. (2012) have shown that the abnormal megakaryopoiesis characterizing ET accounts for a shorter lasting antiplatelet effect of low-dose aspirin through faster renewal of platelet COX-1, and impaired platelet inhibition can be rescued in most patients by modulating the aspirin dosing interval rather than the dose.

Based on these proof-of-concept findings, Rocca et al. (2020) designed a multicentre, randomized, double-blind trial of three low-dose aspirin regimens to optimize antiplatelet therapy in ET. Patients on chronic once-daily low-dose aspirin were randomized (1:1:1) to receive 100 mg of aspirin 1, 2 or 3 times daily for 2 weeks, with repeated measurements of serum TXB_2 and urinary TXM. The study results showed that the currently recommended aspirin regimen of 75 to 100 mg once daily for cardiovascular prophylaxis is largely inadequate in reducing in vivo platelet activation in the vast majority of ET patients (Rocca et al., 2020). The antiplatelet response to low-dose aspirin could be markedly improved by shortening the dosing interval to 12 h, with no further improvement with a reduction to 8 h (Rocca et al., 2020).

The results of this study provide the basis for a personalized approach to antiplatelet therapy in ET and the rationale for revising current recommendations in this setting.

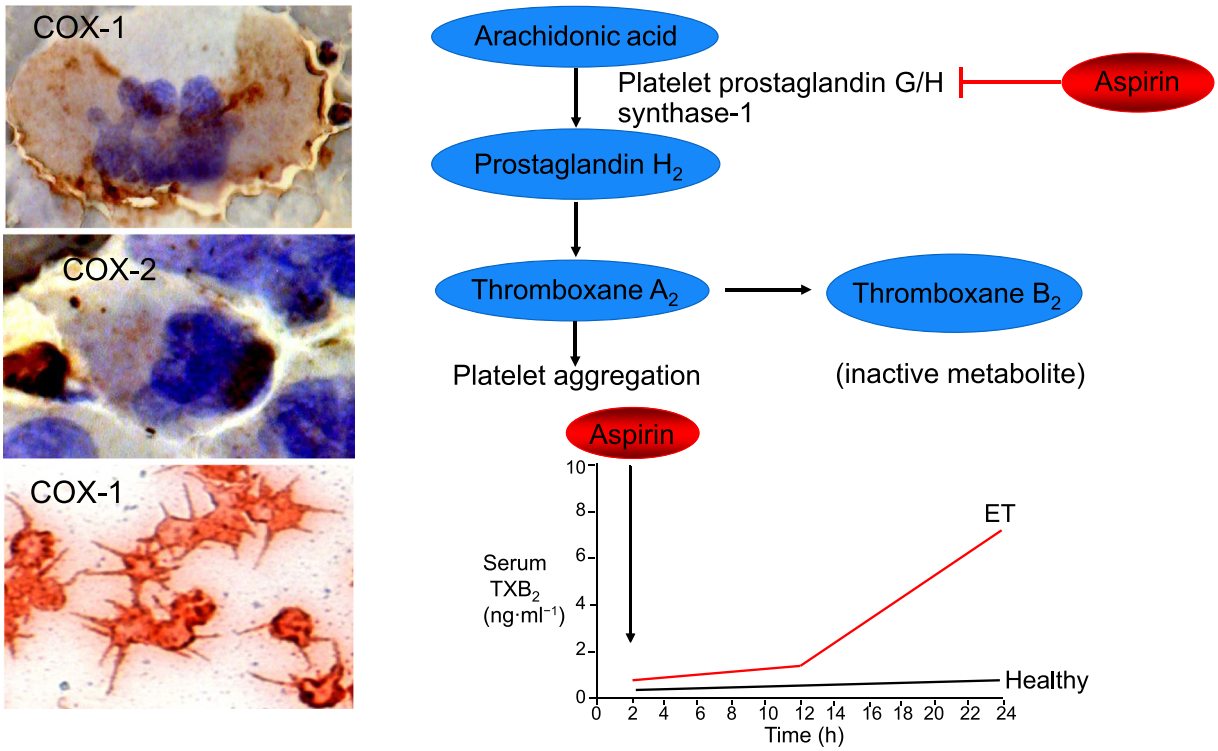

FIGURE 6 Aspirin-resistant thromboxane (TX) biosynthesis in essential thrombocythemia (ET) is explained by accelerated renewal of the drug target. Under conditions of normal megakaryopoiesis, low-dose aspirin acetylates cyclooxygenase (COX)-isozymes in both circulating platelets (COX-1) and bone-marrow megakaryocytes (COX-1 and COX-2), but negligible amounts of unacetylated enzymes are resynthesized within the 24-h dosing interval. This pharmacodynamic pattern is associated with virtually complete suppression of platelet TXA_2 production in the peripheral blood throughout the dosing interval. Under conditions of abnormal megakaryopoiesis, such as in ET, an accelerated rate of COX-isozyme resynthesis is biologically plausible in bone-marrow megakaryocytes, accompanied by faster release of immature platelets with unacetylated enzyme(s) during the aspirin dosing interval, and in particular between 12 and 24 h after dosing. This pharmacodynamic pattern is associated with incomplete suppression of platelet TXA_2 production in the peripheral blood and time-dependent recovery of TXA_2-dependent platelet function during the 24-h dosing interval. Immunohistochemistry panels depicting COX-isozyme expression in bone-marrow megakaryocytes (top and middle panels) and blood platelets (lower panel) are a courtesy of Prof. Bianca Rocca. Pharmacodynamic data are from Pascale et al. (2012).

9 | ASPIRIN AND CANCER: FROM CLINICAL OBSERVATIONS TO MECHANISTIC STUDIES AND BACK TO CLINICAL TRIALS

In 1988, Gabriel Kune, a surgeon at the University of Melbourne, reported associations between colorectal cancer (CRC) risk and several chronic illnesses, operations and various medications in 715 CRC cases and 727 age/sex-matched controls within a population-based observational study (Kune et al., 1988). Among other findings, there was a statistically significant deficit among cases in the use of aspirin and aspirin-containing medications, and this was consistent for both colon and rectal cancer and for both males and females (Kune et al., 1988). In the discussion of their findings, the authors prophetically stated 'Aspirin is now widely used in the chemoprophylaxis of cardiovascular disease and may also be useful in a similar way in the prevention of colorectal cancer and perhaps also of other cancers' (Kune et al., 1988). The results of the Melbourne CRC Study have since been confirmed by numerous observational studies suggesting a 20% to 40% reduced risk of colorectal and other digestive tract cancers associated with aspirin use (reviewed by Bosetti et al., 2020; Patrignani & Patrono, 2016; Ricciotti & FitzGerald, 2021).

Post hoc analyses of long-term (up to 20 years) follow-up of randomized clinical trials of aspirin for cardiovascular prevention, performed by Peter Rothwell and colleagues at the University of Oxford, have yielded results largely consistent with the observational studies (Algra & Rothwell, 2012; Flossmann & Rothwell, 2007; Rothwell et al., 2010). Additional evidence supporting a chemopreventive effect of aspirin against CRC derives from (i) prospective, 20-year follow-up of the Women's Health Study (WHS), the largest placebo-controlled primary prevention trial of low-dose (100 mg on alternate days) aspirin (Cook et al., 2013); (ii) an IPD meta-analysis of four placebo-controlled, randomized trials of aspirin (81 to 325 mg once daily for about 3 years) in subjects with recent histories of sporadic colorectal adenoma or large-bowel cancer that demonstrated a 17% relative risk reduction in any adenoma recurrence and a 28% reduction in the recurrence of advanced lesions, with no apparent dose dependence of the chemopreventive effect within the fourfold range of daily doses used in these trials (Cole et al., 2009); and (iii) CAPP2 (Colorectal

Adenoma/Carcinoma Prevention Programme 2), a placebo-controlled randomized trial of aspirin 600 mg daily in patients with Lynch syndrome, the major form of hereditary CRC (Burn et al., 2008). Although there was no detectable clinical benefit during the scheduled treatment period among carriers of a mutation for Lynch syndrome who received aspirin for up to 4 years (Burn et al., 2008), a statistically significant reduction in cancer incidence was found after a mean follow-up period of 56 months for participants completing 2 years of intervention (Burn et al., 2011), consistent with aspirin preventing an early event(s) in colorectal carcinogenesis, as suggested by the adenoma recurrence prevention trials (Cole et al., 2009). During a planned 10-year follow-up of the same patients, aspirin significantly reduced the risk of CRC by 35%, with no evidence of attenuation of its protective effect up to 20 years (Figure 7). Interestingly, the Kaplan–Meier analyses of time to first CRC in randomized trials with serious vascular events as the primary endpoint (United Kingdom Transient Ischaemic Attack [UK-TIA] Aspirin Trial; Thrombosis Prevention Trial [TPT]; and Swedish Aspirin Low Dose Trial [SALT]), and in one trial with cancer as a secondary endpoint (WHS), show that the protective effect of aspirin takes 5 to 10 years to become apparent in younger healthy women and older high-risk patients, similarly to the time course of its chemopreventive effect in young adult carriers of a germline DNA mismatch repair gene defect (Figure 7) (Burn et al., 2020; Cook et al., 2013; Rothwell et al., 2010).

Is there a biologically plausible mechanism to explain the apparent chemopreventive effect of aspirin against CRC? Because this effect is shared by other traditional NSAIDs and coxibs, (though limited to secondary prevention of sporadic colorectal adenoma recurrence), it was thought initially that it might be related to reduced intestinal inflammation and other COX-2-dependent proliferative and pro-angiogenic mechanisms (reviewed by Thun et al., 2002). However, in reviewing the limited observational evidence available in 2001, we argued that 'The apparent protection against both colorectal adenoma and carcinoma, recently described in association with once-a-day aspirin

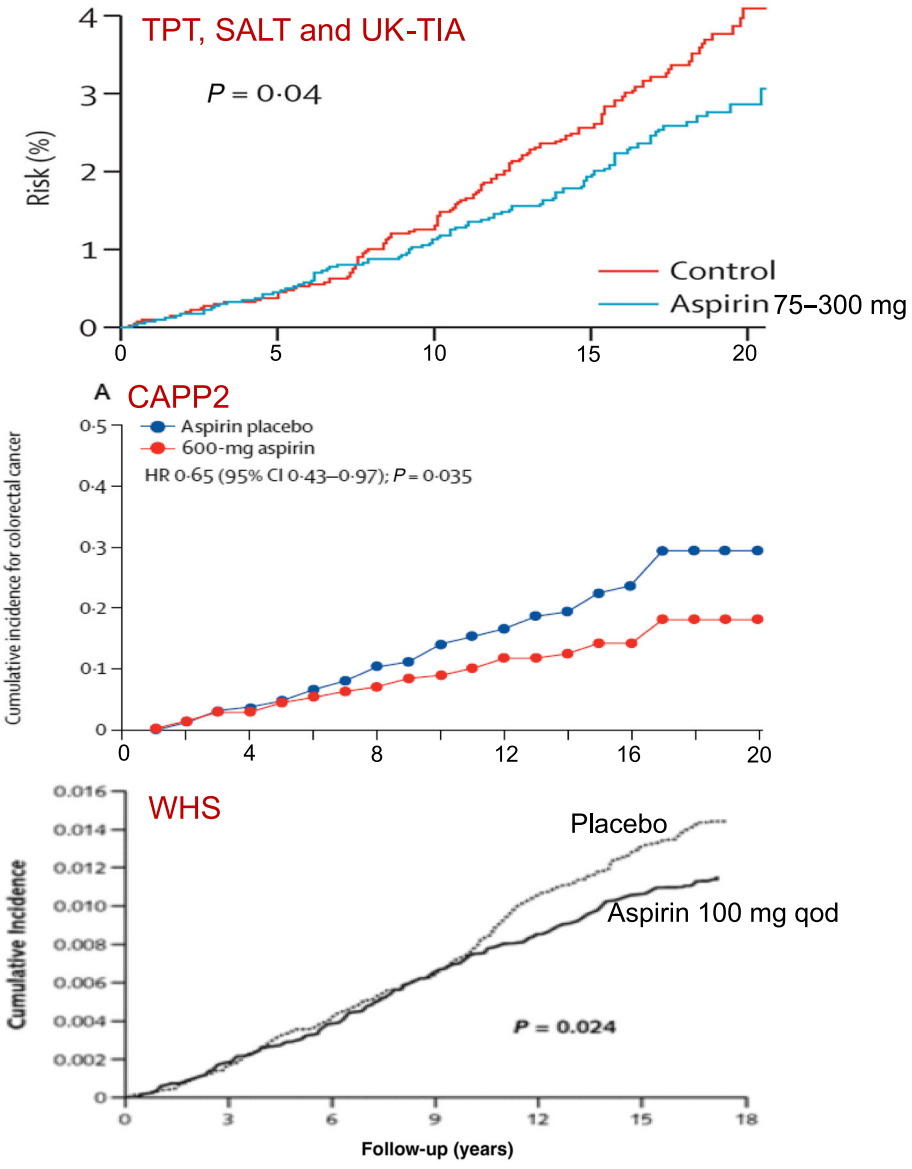

FIGURE 7 Effect of aspirin on long-term risk of colorectal cancer. The effect of aspirin (75–300 mg daily) assignment compared with control, on subsequent incidence of colorectal cancer in all randomized patients (n = 8073) in the Thrombosis Prevention Trial (TPT), the Swedish Aspirin Low Dose Trial (SALT) and the United Kingdom Transient Ischaemic Attack (UK-TIA) Aspirin Trial (lower dose aspirin versus control) (upper panel); time to first colorectal cancer in all Colorectal Adenoma/Carcinoma Prevention Programme 2 (CAPP2) study participants (n = 861) followed up for 10 years and for 20 years in England, Finland and Wales (middle panel); cumulative incidence of colorectal cancer from time of randomization by randomized aspirin (100 mg every other day) assignment in 39,876 women aged 45 and over in the Women's Health Study (WHS), 33,682 of whom continued observational follow-up, with *P*-value from log-rank test (lower panel). Reproduced from Rothwell et al. (2010), Burn et al. (2020) and Cook et al. (2013).

regimens (García Rodríguez & Huerta-Alvarez, 2000; García Rodríguez & Huerta-Alvarez, 2001) raises the intriguing possibility that permanent inactivation of platelet COX-1 restores antitumor reactivity. This working hypothesis is apparently at odds with the proposed COX-2 dependence of early intestinal carcinogenesis, but the models could be reconciled if it could be shown that activated platelets signal COX-2 upregulation in one or more cell types involved in tumor induction and/or angiogenesis. Such a platelet-dependent effect might work through paracrine mediators, either lipids or proteins' (Patrono et al., 2001). This working hypothesis (Figure 8) was largely based on the lack of a dose–response relationship in the observed association of aspirin use and reduced risk of CRC, as well as on the established role of platelets in tissue repair (Patrono et al., 2001). Evidence accumulated over the next 20 years is consistent with this hypothesis by showing that (i) reduced incidence and mortality due to CRC were shown at daily doses as low as 75 mg in the SALT and TPT trials (Rothwell et al., 2010); (ii) the apparent chemopreventive effect of aspirin was not dose dependent, that is, 4- to 16-fold higher doses, as used in the UK-TIA trial, were not more effective than lower doses (Rothwell et al., 2010); (iii) chemoprevention was apparent in men at high cardiovascular risk recruited in TPT and treated with a 75-mg controlled-release aspirin formulation developed to maximize cumulative inhibition of platelet COX-1 in the pre-hepatic circulation (Pedersen & FitzGerald, 1984) and minimize inhibition of COX-2 in the systemic compartment (Clarke et al., 1991); and (iv) in the long-term observational follow-up of the WHS, reduced risk for CRC was reported in apparently healthy women treated with an alternate-day dosing regimen of 100-mg aspirin versus placebo (Cook et al., 2013). Thus, the main features of the chemopreventive effect of aspirin are consistent with the unique characteristics of its platelet COX-1 inhibitory effect, that is, its long-lasting duration and, most importantly, its saturability at low doses (Patrignani et al., 1982; Patrignani et al., 2014).

A three-step model has been proposed (Patrignani & Patrono, 2016; Patrignani & Patrono, 2018; Patrono et al., 2001) to explain the potential contribution of blood platelets to the early stages of colorectal tumorigenesis (Figure 8): (i) Platelets are activated at sites of intestinal mucosal injury and release a variety of lipid and protein mediators involved in tissue repair, that is, TXA_2, ADP, growth and angiogenic factors, and inflammatory cytokines; (ii) when this physiological response is not restricted in time and space, persistent autacoid release from activated platelets may trigger an inflammatory response in adjacent stromal cells leading to COX-2 induction and further release of growth-promoting and pro-angiogenic soluble mediators; and (iii) these events may contribute to the transition from a normal intestinal mucosa to an adenomatous lesion, as suggested by the efficacy of low-dose aspirin in reducing the risk of sporadic colorectal adenoma recurrence (Cole et al., 2009).

Proof of concept of the capacity of platelets to drive intestinal inflammation and COX-2 expression was provided by Paola Patrignani

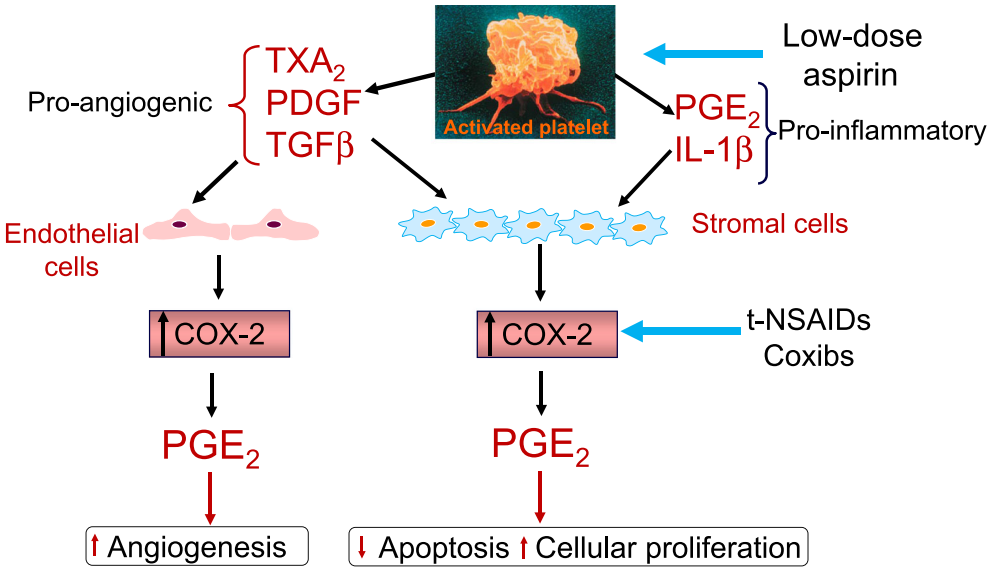

FIGURE 8 The potential role of platelet activation in the early stage of colorectal carcinogenesis. In the first stages of intestinal tumourigenesis, platelets may play a key role, because they are activated in response to intestinal mucosal injury and participate in tissue repair. However, when platelet activation is not controlled in time and space, the same mechanism may contribute to the induction of several signalling pathways through paracrine soluble mediators, such as thromboxane (TX)A_2 and prostaglandin (PG)E_2, growth factors and inflammatory cytokines, in turn inducing cyclooxygenase (COX)-2 expression in adjacent nucleated cells, and an eicosanoid amplification loop promoting cell proliferation and angiogenesis. A sequential involvement of COX-1 (in platelets) and COX-2 (in various nucleated cells) in the early events leading to the transformation of an apparently normal intestinal mucosa into an adenomatous lesion would explain the similar protective effect of low-dose aspirin and COX-2 inhibitors in reducing the recurrence rate of a sporadic colorectal adenoma over the first 3 years of treatment and protecting against cancer development over 5–10 years. This working hypothesis was first articulated by Patrono et al. (2001) and further developed by Patrignani and Patrono (2016, 2018).

and colleagues at the University of Chieti by showing that megakaryocyte/platelet-specific deletion of COX-1 ameliorates dextran sulfate sodium-induced colitis and down-regulates intestinal COX-2 expression in mice (Sacco et al., 2019). Moreover, using xenograft colon cancer models, Cariello et al. (2022) recently reported that platelets from patients with visceral obesity, a known risk factor for CRC, promote colon cancer growth. Visceral obesity had been previously shown to be characterized by persistent TXA_2-dependent platelet activation, driven by inflammatory triggers related to the degree of abdominal adiposity (Davì et al., 2002).

Additional evidence for a chemopreventive effect of low-dose aspirin against CRC (and other cancers) may come over the next 5 years from at least two additional sources: (i) long-term, prospective follow-up of three recent low-dose aspirin trials involving over 47,000 participants, in which cancer incidence was a prespecified secondary endpoint, coordinated by Prof. Peter Rothwell at the University of Oxford; and (ii) a large adjuvant cancer trial, the Add-Aspirin trial, in which the efficacy and safety of aspirin (100 or 300 mg daily) is being tested versus placebo in over 8000 patients with an early-stage, common solid tumour (gastro-oesophageal, colorectal, breast and prostate) who received their primary treatment with curative intent and are being followed for at least 5 years (Joharatnam-Hogan et al., 2019). The trial is coordinated by Prof. Ruth Langley at the University College London, and primary outcome data are expected in 2025–2027.

10 | CONCLUSIONS

The year 2022 marks the 125th anniversary of the first synthesis of acetylsalicylic acid within an industrial environment. Despite this venerable age, aspirin continues to represent the cornerstone of antiplatelet therapy for the acute treatment and long-term prevention of atherothrombosis (Patrono, 2022). The development of a selective antiplatelet regimen of low-dose aspirin has been instrumental in maximizing its antithrombotic efficacy and minimizing its gastrointestinal toxicity. Moreover, its use as an investigative tool was key to expanding our knowledge about the multifaceted roles of platelets in health and disease (Patrono, 2015).

Newer antiplatelet drugs, targeting other mediators of platelet activation, have also been developed during the past 30 years, but none has been convincingly shown to be more effective or safer than aspirin (Patrono et al., 2017). The combination of low-dose aspirin with other antiplatelet (e.g., $P2Y_{12}$ receptor antagonists) or anticoagulant drugs (e.g., factor Xa inhibitors) is now considered a more effective antithrombotic strategy for high-risk patients than replacing aspirin with a newer agent.

The intriguing possibility that long-term platelet inhibition may also interfere with the early stage(s) of colorectal carcinogenesis is currently being investigated, both experimentally and through large, placebo-controlled, randomized clinical trials. The results of these trials will validate or challenge the underlying mechanistic hypothesis and, if successful, provide a rationale for investigating the efficacy and safety of more intensive platelet inhibition through combined therapy.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTIONS

Carlo Patrono: Conceptualization; drafting and revising the manuscript.

REFERENCES

Algra, A. M., & Rothwell, P. M. (2012). Effects of regular aspirin on long-term cancer incidence and metastasis: A systematic comparison of evidence from observational studies versus randomised trials. *The Lancet Oncology*, *13*, 518–527. https://doi.org/10.1016/S1470-2045(12)70112-2

Antithrombotic Trialists' Collaboration. (2002). Collaborative meta-analysis of randomised trials of antiplatelet therapy for prevention of death, myocardial infarction, and stroke in high risk patients. *BMJ*, *324*, 71–86. https://doi.org/10.1136/bmj.324.7329.71

Bertelé, V., Falanga, A., Tomasiak, M., Dejana, E., Cerletti, C., & de Gaetano, G. (1983). Platelet thromboxane synthetase inhibitors with low doses of aspirin: Possible resolution of the "aspirin dilemma". *Science*, *220*, 517–519. https://doi.org/10.1126/science.6682245

Born, G., & Patrono, C. (2006). Antiplatelet drugs. *British Journal of Pharmacology*, *147*(Suppl 1), S241–S251. https://doi.org/10.1038/sj.bjp.0706401

Born, G. V. R. (1962a). Quantitative investigations into the aggregation of blood platelets. *The Journal of Physiology*, *162*, 7–68.

Born, G. V. R. (1962b). Aggregation of blood platelets by adenosine diphosphate and its reversal. *Nature*, *194*, 927–929. https://doi.org/10.1038/194927b0

Born, G. V. R., & Cross, M. J. (1962). Inhibition of the aggregation of blood platelets by substances related to adenosine diphosphate. *The Journal of Physiology*, *166*, 29–30.

Born, G. V. R., & Cross, M. J. (1963). The aggregation of blood platelets. *The Journal of Physiology*, *168*, 179–195. https://doi.org/10.1113/jphysiol.1963.sp007185

Born, G. V. R., Honour, A. J., & Mitchell, J. R. A. (1964). Inhibition by adenosine and by 2-chloroadenosine of the formation and embolisation of platelet thrombi. *Nature (London)*, *202*, 761–765. https://doi.org/10.1038/202761a0

Bosetti, C., Santucci, C., Gallus, S., Martinetti, M., & La Vecchia, C. (2020). Aspirin and the risk of colorectal and other digestive tract cancers: An updated meta-analysis to 2019. *Annals of Oncology*, *31*, 558–568. https://doi.org/10.1016/j.annonc.2020.02.012

Breddin, K., Loew, D., Lechner, K., Oberla, K., & Walter, E. (1980). The German-Austrian aspirin trial: A comparison of acetylsalicylic acid, placebo and phenprocoumon in secondary prevention of myocardial

infarction. On behalf of the German-Austrian Study Group. *Circulation*, *62*, V63–V72.

Burch, J. W., Baenziger, N. L., Stanford, N., & Majerus, P. W. (1978). Sensitivity of fatty acid cyclooxygenase from human aorta to acetylation by aspirin. *Proceedings of the National Academy of Sciences of the United States of America*, *75*, 5181–5184. https://doi.org/10.1073/pnas.75.10.5181

Burch, J. W., Stanford, N., & Majerus, P. W. (1978). Inhibition of platelet prostaglandin synthetase by oral aspirin. *The Journal of Clinical Investigation*, *61*, 314–319. https://doi.org/10.1172/JCI108941

Burn, J., Bishop, D. T., Mecklin, J. P., Macrae, F., Möslein, G., Olschwang, S., Bisgaard, M. L., Ramesar, R., Eccles, D., Maher, E. R., Bertario, L., Jarvinen, H. J., Lindblom, A., Evans, D. G., Lubinski, J., Morrison, P. J., Ho, J. W. C., Vasen, H. F. A., Side, L., … for the CAPP2 Investigators. (2008). Effect of aspirin or resistant starch on colorectal neoplasia in the Lynch syndrome. *The New England Journal of Medicine*, *359*, 2567–2578. https://doi.org/10.1056/NEJMoa0801297

Burn, J., Gerdes, A. M., Macrae, F., Mecklin, J. P., Moeslein, G., Olschwang, S., Eccles, D., Evans, D. G., Maher, E. R., Bertario, L., Bisgaard, M. L., Dunlop, M. G., Ho, J. W., Hodgson, S. V., Lindblom, A., Lubinski, J., Morrison, P. J., Murday, V., Ramesar, R., … for the CAPP2 Investigators. (2011). Long-term effect of aspirin on cancer risk in carriers of hereditary colorectal cancer: An analysis from the CAPP2 randomised controlled trial. *Lancet*, *378*, 2081–2087. https://doi.org/10.1016/S0140-6736(11)61049-0

Burn, J., Sheth, H., Elliott, F., Reed, L., Macrae, F., Mecklin, J. P., Möslein, G., McRonald, F. E., Bertario, L., Evans, D. G., Gerdes, A. M., Ho, J. W. C., Lindblom, A., Morrison, P. J., Rashbass, J., Ramesar, R., Seppälä, T., Thomas, H. J. W., Pylvänäinen, K., … for the CAPP2 Investigators. (2020). Cancer prevention with aspirin in hereditary colorectal cancer (Lynch syndrome), 10-year follow-up and registry-based 20-year data in the CAPP2 study: A double-blind, randomised, placebo-controlled trial. *Lancet*, *395*, 1855–1863. https://doi.org/10.1016/S0140-6736(20)30366-4

Cairns, J. A., Gent, M., Singer, J., Finnie, K. J., Froggatt, G. M., Holder, D. A., Jablonsky, G., Kostuk, W. J., Melendez, L. J., Myers, M. G., Sackett, D. L., Sealey, B. J., & Tanser, P. H. (1985). Aspirin, sulfinpyrazone, or both in unstable angina. Results of a Canadian multicenter trial. *The New England Journal of Medicine*, *313*, 1369–1375. https://doi.org/10.1056/NEJM198511283132201

Cariello, M., Piccinin, E., Pasculli, E., Arconzo, M., Zerlotin, R., D'Amore, S., Mastropasqua, F., Peres, C., Graziano, G., Villani, G., Pesole, G., & Moschetta, A. (2022). Platelets from patients with visceral obesity promote colon cancer growth. *Communications Biology*, *5*, 553. https://doi.org/10.1038/s42003-022-03486-7

Catella-Lawson, F., Reilly, M. P., Kapoor, S. C., Cucchiara, A. J., DeMarco, S., Tournier, B., Vyas, S. N., & FitzGerald, G. A. (2001). Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *The New England Journal of Medicine*, *345*, 1809–1817. https://doi.org/10.1056/NEJMoa003199

Cavalca, V., Rocca, B., Veglia, F., Petrucci, G., Porro, B., Myasoedova, V., de Cristofaro, R., Turnu, L., Bonomi, A., Songia, P., Cavallotti, L., Zanobini, M., Camera, M., Alamanni, F., Parolari, A., Patrono, C., & Tremoli, E. (2017). On-pump cardiac surgery enhances platelet renewal and impairs aspirin pharmacodynamics: Effects of improved dosing regimens. *Clinical Pharmacology and Therapeutics*, *102*, 849–858. https://doi.org/10.1002/cpt.702

Chesebro, J. H., Fuster, V., Elveback, L. R., Clements, I. P., Smith, H. C., Holmes, D. R. Jr., Bardsley, W. T., Pluth, J. R., Wallace, R. B., Puga, F. J., Orszulak, T. A., Piehler, J. M., Danielson, G. K., Schaff, H. V., & Frye, R. L. (1984). Effect of dipyridamole and aspirin on late vein-graft patency after coronary bypass operations. *The New England Journal of Medicine*, *310*, 209–214. https://doi.org/10.1056/NEJM198401263100401

Clarke, R. J., Mayo, G., Price, P., & FitzGerald, G. A. (1991). Suppression of thromboxane A_2 but not of systemic prostacyclin by controlled-release aspirin. *The New England Journal of Medicine*, *325*, 1137–1141. https://doi.org/10.1056/NEJM199110173251605

Cole, B. F., Logan, R. F., Halabi, S., Benamouzig, R., Sandler, R. S., Grainge, M. J., Chaussade, S., & Baron, J. A. (2009). Aspirin for the chemoprevention of colorectal adenomas: Meta-analysis of the randomized trials. *Journal of the National Cancer Institute*, *101*, 256–266. https://doi.org/10.1093/jnci/djn485

Cook, N. R., Lee, I. M., Zhang, S. M., Moorthy, M. V., & Buring, J. E. (2013). Alternate-day, low-dose aspirin and cancer risk: Long-term observational follow-up of a randomized trial. *Annals of Internal Medicine*, *159*, 77–85. https://doi.org/10.7326/0003-4819-159-2-201307160-00002

Coxib and traditional NSAID Trialists' (CNT) Collaboration, Bhala, N., Emberson, J., Merhi, A., Abramson, S., Arber, N., Baron, J. A., Bombardier, C., Cannon, C., Farkouh, M. E., FitzGerald, G., Goss, P., Halls, H., Hawk, E., Hawkey, C., Hennekens, C., Hochberg, M., Holland, L. E., Kearney, P. M., … Baigent, C. (2013). Vascular and upper gastrointestinal effects of non-steroidal anti-inflammatory drugs: Meta-analyses of individual participant data from randomised trials. *Lancet*, *382*, 769–779. https://doi.org/10.1016/S0140-6736(13)60900-9

Davì, G., Guagnano, M. T., Ciabattoni, G., Basili, S., Falco, A., Marinopiccoli, M., Nutini, M., Sensi, S., & Patrono, C. (2002). Platelet activation in obese women: Role of inflammation and oxidant stress. *Jama*, *288*, 2008–2014. https://doi.org/10.1001/jama.288.16.2008

Davì, G., & Patrono, C. (2007). Platelet activation and atherothrombosis. *The New England Journal of Medicine*, *357*, 2482–2494. https://doi.org/10.1056/NEJMra071014

de Caterina, R., Giannessi, D., Bernini, W., Gazzetti, P., Michelassi, C., L'Abbate, A., donato, l., Patrignanl, p., Fllabozzl, P., & Patrono, C. (1985). Low-dose aspirin in patients recovering from myocardial infarction. Evidence for a selective inhibition of thromboxane-related platelet function. *European Heart Journal*, *6*(5), 409–417. https://doi.org/10.1093/oxfordjournals.eurheartj.a061879

Dragani, A., Pascale, S., Recchiuti, A., Mattoscio, D., Lattanzio, S., Petrucci, G., Mucci, L., Ferrante, E., Habib, A., Ranelletti, F. O., Ciabattoni, G., Davì, G., Patrono, C., & Rocca, B. (2010). The contribution of cyclooxygenase-1 and -2 to persistent thromboxane biosynthesis in aspirin-insensitive essential thrombocythemia: Implications for antiplatelet therapy. *Blood*, *115*, 1054–1061. https://doi.org/10.1182/blood-2009-08-236679

Evans, G., Packham, M. A., Nishizawa, E. E., Mustard, J. F., & Murphy, E. A. (1968). The effect of acetylsalicyclic acid on platelet function. *The Journal of Experimental Medicine*, *128*, 877–894. https://doi.org/10.1084/jem.128.5.877

Ferreira, S. H., Moncada, S., & Vane, J. R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. *Nature: New Biology*, *231*, 237–239. https://doi.org/10.1038/newbio231237a0

FitzGerald, G. A., Brash, A. R., Falardeau, P., & Oates, J. A. (1981). Estimated rate of prostacyclin secretion into the circulation of normal man. *The Journal of Clinical Investigation*, *68*, 1272–1276. https://doi.org/10.1172/jci110373

FitzGerald, G. A., Oates, J. A., Hawiger, J., Maas, R. L., Roberts, L. J. 2nd, Lawson, J. A., & Brash, A. R. (1983). Endogenous biosynthesis of prostacyclin and thromboxane and platelet function during chronic administration of aspirin in man. *The Journal of Clinical Investigation*, *71*(3), 676–688. https://doi.org/10.1172/jci110814

FitzGerald, G. A., & Patrono, C. (2001). The coxibs, selective inhibitors of cyclooxygenase-2. *The New England Journal of Medicine*, *345*, 433–442. https://doi.org/10.1056/NEJM200108093450607

FitzGerald, G. A., Pedersen, A. K., & Patrono, C. (1983). Analysis of prostacyclin and thromboxane biosynthesis in cardiovascular

disease. *Circulation*, *67*, 1174–1177. https://doi.org/10.1161/01.cir.67.6.1174

Flossmann, E., Rothwell, P. M., & for the British Doctors Aspirin Trial and the UK-TIA Aspirin Trial. (2007). Effect of aspirin on long-term risk of colorectal cancer: Consistent evidence from randomised and observational studies. *Lancet*, *369*, 1603–1613. https://doi.org/10.1016/S0140-6736(07)60747-8

García Rodríguez, L. A., & Huerta-Alvarez, C. (2000). Reduced incidence of colorectal adenoma among long-term users of nonsteroidal antiinflammatory drugs: A pooled analysis of published studies and a new population-based study. *Epidemiology*, *11*, 376–381. https://doi.org/10.1097/00001648-200007000-00003

García Rodríguez, L. A., & Huerta-Alvarez, C. (2001). Reduced risk of colorectal cancer among long-term users of aspirin and non-aspirin nonsteroidal anti-inflammatory drugs. *Epidemiology*, *12*, 88–93. https://doi.org/10.1097/00001648-200101000-00015

Gast, L. F. (1964). Influence of aspirin on haemostatic parameters. *Annals of the Rheumatic Diseases*, *23*, 500–504. https://doi.org/10.1136/ard.23.6.500

Grosser, T., Fries, S., Lawson, J. A., Kapoor, S. C., Grant, G. R., & FitzGerald, G. A. (2013). Drug resistance and pseudoresistance: An unintended consequence of enteric coating aspirin. *Circulation*, *127*, 377–385. https://doi.org/10.1161/CIRCULATIONAHA.112.117283

Grosser, T., Yu, Y., & FitzGerald, G. A. (2010). Emotion recollected in tranquility: Lessons learned from the COX-2 saga. *Annual Review of Medicine*, *61*, 17–33. https://doi.org/10.1146/annurev-med-011209-153129

Gum, P. A., Kottke-Marchant, K., Welsh, P. A., White, J., & Topol, E. J. (2003). A prospective, blinded determination of the natural history of aspirin resistance among stable patients with cardiovascular disease. *Journal of the American College of Cardiology*, *41*, 961–965. https://doi.org/10.1016/s0735-1097(02)03014-0

Hamberg, M., & Samuelsson, B. (1973). Detection and isolation of an endoperoxide intermediate in prostaglandin biosynthesis. *Proceedings of the National Academy of Sciences of the United States of America*, *70*, 899–903. https://doi.org/10.1073/pnas.70.3.899

Hamberg, M., & Samuelsson, B. (1974). Prostaglandin endoperoxides. Novel transformations of arachidonic acid in human platelets. *Proceedings of the National Academy of Sciences of the United States of America*, *71*, 3400–3404. https://doi.org/10.1073/pnas.71.9.3400

Hamberg, M., Svensson, J., & Samuelsson, B. (1974). Prostaglandin endoperoxides. A new concept concerning the mode of action and release of prostaglandins. *Proceedings of the National Academy of Sciences of the United States of America*, *71*, 3824–3828. https://doi.org/10.1073/pnas.71.10.3824

Hamberg, M., Svensson, J., & Samuelsson, B. (1975). Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. *Proceedings of the National Academy of Sciences of the United States of America*, *72*, 2994–2998. https://doi.org/10.1073/pnas.72.8.2994

Hamberg, M., Svensson, J., Wakabayashi, T., & Samuelsson, B. (1974). Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. *Proceedings of the National Academy of Sciences of the United States of America*, *71*, 345–349. https://doi.org/10.1073/pnas.71.2.345

Harter, H. R., Burch, J. W., Majerus, P. W., Stanford, N., Delmez, J. A., Anderson, C. B., & Weerts, C. A. (1979). Prevention of thrombosis in patients on hemodialysis by low-dose aspirin. *The New England Journal of Medicine*, *301*, 577–579. https://doi.org/10.1056/NEJM197909133011103

Hawkins, D., Pinckard, R. N., Crawford, I. P., & Farr, R. S. (1969). Structural changes in human serum albumin induced by ingestion of acetylsalicylic acid. *The Journal of Clinical Investigation*, *48*, 536–542. https://doi.org/10.1172/JCI106011

ISIS-2 (Second International Study of Infarct Survival) Collaborative Group. (1988). Randomised trial of intravenous streptokinase, oral aspirin, both, or neither among 17,187 cases of suspected acute myocardial infarction. *Lancet*, *2*, 349–360. https://doi.org/10.1016/S0140-6736(88)92833-4

Joharatnam-Hogan, N., Cafferty, F., Hubner, R., Swinson, D., Sothi, S., Gupta, K., Falk, S., Patel, K., Warner, N., Kunene, V., Rowley, S., Khabra, K., Underwood, T., Jankowski, J., Bridgewater, J., Crossley, A., Henson, V., Berkman, L., Gilbert, D., … Steele, R. J. C. (2019). Aspirin as an adjuvant treatment for cancer: Feasibility results from the Add-Aspirin randomised trial. *The Lancet Gastroenterology & Hepatology*, *4*(11), 854–862. https://doi.org/10.1016/S2468-1253(19)30289-4

Jones, W. S., Mulder, H., Wruck, L. M., Pencina, M. J., Kripalani, S., Muñoz, D., Crenshaw, D. L., Effron, M. B., Re, R. N., Gupta, K., Anderson, R. D., Pepine, C. J., Handberg, E. M., Manning, B. R., Jain, S. K., Girotra, S., Riley, D., DeWalt, D., Whittle, J., … ADAPTABLE Team. (2021). Comparative effectiveness of aspirin dosing in cardiovascular disease. *The New England Journal of Medicine*, *384*, 1981–1990. https://doi.org/10.1056/NEJMoa2102137

Kune, G. A., Kune, S., & Watson, L. F. (1988). Colorectal cancer risk, chronic illnesses, operations, and medications: Case control results from the Melbourne Colorectal Cancer Study. *Cancer Research*, *48*, 4399–4404.

Li, X., Fries, S., Li, R., Lawson, J. A., Propert, K. J., Diamond, S. L., Blair, I. A., FitzGerald, G. A., & Grosser, T. (2014). Differential impairment of aspirin-dependent platelet cyclooxygenase acetylation by nonsteroidal antiinflammatory drugs. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 16830–16835. https://doi.org/10.1073/pnas.1406997111

Marcus, A. J. (1983). Aspirin as an antithrombotic medication. *The New England Journal of Medicine*, *309*, 1515–1517. https://doi.org/10.1056/NEJM198312153092410

McAdam, B. F., Catella-Lawson, F., Mardini, I. A., Kapoor, S., Lawson, J. A., & Fitz Gerald, G. A. (1999). Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2. *Proceedings of the National Academy of Sciences of the United States of America*, *96*, 272–277. https://doi.org/10.1073/pnas.96.1.272

Moncada, S., & Vane, J. R. (1979). Arachidonic acid metabolites and the interactions between platelets and blood-vessel walls. *The New England Journal of Medicine*, *300*, 1142–1147. https://doi.org/10.1056/NEJM197905173002006

O'Brien, J. R. (1968). Effects of salicylates on human platelets. *Lancet*, *1*, 779–783. https://doi.org/10.1016/s0140-6736(68)92228-9

Pascale, S., Petrucci, G., Dragani, A., Habib, A., Zaccardi, F., Pagliaccia, F., Pocaterra, D., Ragazzoni, E., Rolandi, G., Rocca, B., & Patrono, C. (2012). Aspirin-insensitive thromboxane biosynthesis in essential thrombocythemia is explained by accelerated renewal of the drug target. *Blood*, *119*(15), 3595–3603. https://doi.org/10.1182/blood-2011-06-359224

Patrignani, P., Filabozzi, P., & Patrono, C. (1982). Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *The Journal of Clinical Investigation*, *69*, 1366–1372. https://doi.org/10.1172/jci110576

Patrignani, P., & Patrono, C. (2016). Aspirin and cancer. *Journal of the American College of Cardiology*, *68*, 967–976. https://doi.org/10.1016/j.jacc.2016.05.083

Patrignani, P., & Patrono, C. (2018). Aspirin, platelet inhibition and cancer prevention. *Platelets*, *29*, 779–785. https://doi.org/10.1080/09537104.2018.1492105

Patrignani, P., Tacconelli, S., Piazuelo, E., di Francesco, L., Dovizio, M., Sostres, C., Marcantoni, E., Guillem-Llobat, P., del Boccio, P., Zucchelli, M., Patrono, C., & Lanas, A. (2014). Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and

traditional indirect biomarkers of drug action. *Journal of Thrombosis and Haemostasis*, *12*(8), 1320–1330. https://doi.org/10.1111/jth.12637

Patrono, C. (1984). The aspirin dilemma revisited. *The New England Journal of Medicine*, *310*, 1326–1327. https://doi.org/10.1056/NEJM198405173102014

Patrono, C. (1994). Aspirin as an antiplatelet drug. *The New England Journal of Medicine*, *330*, 1287–1294. https://doi.org/10.1056/NEJM199405053301808

Patrono, C. (2015). The multifaceted clinical read-outs of platelet inhibition by low-dose aspirin. *Journal of the American College of Cardiology*, *66*, 74–85. https://doi.org/10.1016/j.jacc.2015.05.012

Patrono, C. (2016). Cardiovascular effects of cyclooxygenase-2 inhibitors: A mechanistic and clinical perspective. *British Journal of Clinical Pharmacology*, *82*, 957–964. https://doi.org/10.1111/bcp.13048

Patrono, C. (2022). Aspirin: 1A @ 125. *European Heart Journal*, *43*, 3194–3195. https://doi.org/10.1093/eurheartj/ehac416

Patrono, C., & Baigent, C. (2014). Nonsteroidal anti-inflammatory drugs and the heart. *Circulation*, *129*, 907–916. https://doi.org/10.1161/CIRCULATIONAHA.113.004480

Patrono, C., Ciabattoni, G., Greco, F., & Grossi-Belloni, D. (1976). Comparative evaluation of the inhibitory effects of aspirin-like drugs on prostaglandin production by human platelets and synovial tissue. *Advances in Prostaglandin and Thromboxane Research*, *1*, 125–131. PMID: 826138

Patrono, C., Ciabattoni, G., Patrignani, P., Pugliese, F., Filabozzi, P., Catella, F., Davì, G., & Forni, L. (1985). Clinical pharmacology of platelet cyclooxygenase inhibition. *Circulation*, *72*(6), 1177–1184. https://doi.org/10.1161/01.cir.72.6.1177

Patrono, C., Ciabattoni, G., Pinca, E., Pugliese, F., Castrucci, G., de Salvo, A., Satta, M. A., & Peskar, B. A. (1980). Low dose aspirin and inhibition of thromboxane B2 production in healthy subjects. *Thrombosis Research*, *17*(3-4), 317–327. https://doi.org/10.1016/0049-3848(80)90066-3

Patrono, C., Ciabattoni, G., Pugliese, F., Pierucci, A., Blair, I. A., & FitzGerald, G. A. (1986). Estimated rate of thromboxane secretion into the circulation of normal man. *The Journal of Clinical Investigation*, *77*, 590–594. https://doi.org/10.1172/JCI112341

Patrono, C., Coller, B., Dalen, J. E., Fuster, V., Gent, M., Harker, L. A., Hirsh, J., & Roth, G. (1998). Platelet-active drugs: The relationships among dose, effectiveness, and side effects. *Chest*, *114*, 470S–488S. https://doi.org/10.1378/chest.114.5_supplement.470s

Patrono, C., García Rodríguez, L. A., Landolfi, R., & Baigent, C. (2005). Low-dose aspirin for the prevention of atherothrombosis. *The New England Journal of Medicine*, *353*, 2373–2383. https://doi.org/10.1056/NEJMra052717

Patrono, C., Morais, J., Baigent, C., Collet, J. P., Fitzgerald, D., Halvorsen, S., Rocca, B., Siegbahn, A., Storey, R. F., & Vilahur, G. (2017). Antiplatelet agents for the treatment and prevention of coronary atherothrombosis. *Journal of the American College of Cardiology*, *70*, 1760–1776. https://doi.org/10.1016/j.jacc.2017.08.037

Patrono, C., Patrignani, P., & García Rodríguez, L. A. (2001). Cyclooxygenase-selective inhibition of prostanoid formation: Transducing biochemical selectivity into clinical read-outs. *The Journal of Clinical Investigation*, *108*, 7–13. https://doi.org/10.1172/JCI13418

Patrono, C., Pugliese, F., Ciabattoni, G., Patrignani, P., Maseri, A., Chierchia, S., Peskar, B. A., Cinotti, G. A., Simonetti, B. M., & Pierucci, A. (1982). Evidence for a direct stimulatory effect of prostacyclin on renin release in man. *The Journal of Clinical Investigation*, *69*, 231–239. https://doi.org/10.1172/jci110435

Patrono, C., & Rocca, B. (2007). Drug insight: Aspirin resistance—Fact or fashion? *Nature Clinical Practice. Cardiovascular Medicine*, *4*, 42–50. https://doi.org/10.1038/ncpcardio0728

Patrono, C., Rocca, B., & De Stefano, V. (2013). Platelet activation and inhibition in polycythemia vera and essential thrombocythemia. *Blood*, *121*, 1701–1711. https://doi.org/10.1182/blood-2012-10-429134

Pedersen, A. K., & FitzGerald, G. A. (1984). Dose-related kinetics of aspirin: Presystemic acetylation of platelet cyclo-oxygenase. *The New England Journal of Medicine*, *311*, 1206–1211. https://doi.org/10.1056/NEJM198411083111902

Pinckard, R. N., Hawkins, D., & Farr, R. S. (1968). In vitro acetylation of plasma proteins, enzymes and DNA by aspirin. *Nature*, *219*, 68–69. https://doi.org/10.1038/219068a0

Piper, P. J., & Vane, J. R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. *Nature (London)*, *223*, 29–35. https://doi.org/10.1038/223029a0

Quick, A. J. (1966). Salicylates and bleeding: The aspirin tolerance test. *The American Journal of the Medical Sciences*, *252*, 265–269. https://doi.org/10.1097/00000441-196609000-00003

Reilly, I. A., & FitzGerald, G. A. (1987). Inhibition of thromboxane formation in vivo and ex vivo: Implications for therapy with platelet inhibitory drugs. *Blood*, *69*, 180–186. https://doi.org/10.1182/blood.V69.1.180.180

Ricciotti, E., & FitzGerald, G. A. (2021). Aspirin in the prevention of cardiovascular disease and cancer. *Annual Review of Medicine*, *72*, 473–495. https://doi.org/10.1146/annurev-med-051019-102940

Roberts, L. J. II, Sweetman, B. J., & Oates, J. A. (1981). Metabolism of thromboxane B_2 in man. Identification of twenty urinary metabolites. *The Journal of Biological Chemistry*, *256*, 8384–8393. https://doi.org/10.1016/S0021-9258(19)68855-1

Rocca, B., Santilli, F., Pitocco, D., Mucci, L., Petrucci, G., Vitacolonna, E., Lattanzio, S., Mattoscio, D., Zaccardi, F., Liani, R., Vazzana, N., del Ponte, A., Ferrante, E., Martini, F., Cardillo, C., Morosetti, R., Mirabella, M., Ghirlanda, G., Davì, G., & Patrono, C. (2012). The recovery of platelet cyclooxygenase activity explains interindividual variability in responsiveness to low-dose aspirin in patients with and without diabetes. *Journal of Thrombosis and Haemostasis*, *10*(7), 1220–1230. https://doi.org/10.1111/j.1538-7836.2012.04723.x

Rocca, B., Secchiero, P., Ciabattoni, G., Ranelletti, F. O., Catani, L., Guidotti, L., Melloni, E., Maggiano, N., Zauli, G., & Patrono, C. (2002). Cyclooxygenase-2 expression is induced during human megakaryopoiesis and characterizes newly formed platelets. *Proceedings of the National Academy of Sciences of the United States of America*, *99*, 7634–7639. https://doi.org/10.1073/pnas.112202999

Rocca, B., Tosetto, A., Betti, S., Soldati, D., Petrucci, G., Rossi, E., Timillero, A., Cavalca, V., Porro, B., Iurlo, A., Cattaneo, D., Bucelli, C., Dragani, A., di Ianni, M., Ranalli, P., Palandri, F., Vianelli, N., Beggiato, E., Lanzarone, G., … de Stefano, V. (2020). A randomized, double blind trial of three low-dose aspirin regimens to optimize antiplatelet therapy in essential thrombocythemia. *Blood*, *136*, 171–182. https://doi.org/10.1182/blood.2019004596

Roth, G. J., & Majerus, P. W. (1975). The mechanism of the effect of aspirin on human platelets. I. Acetylation of a particulate fraction protein. *The Journal of Clinical Investigation*, *56*, 624–632. https://doi.org/10.1172/JCI108132

Roth, G. J., Stanford, N., & Majerus, P. W. (1975). Acetylation of prostaglandin synthase by aspirin. *Proceedings of the National Academy of Sciences of the United States of America*, *72*, 3073–3076. https://doi.org/10.1073/pnas.72.8.3073

Rothwell, P. M., Wilson, M., Elwin, C.-E., Norrving, B., Algra, A., Warlow, C. P., & Meade, T. W. (2010). Long-term effect of aspirin on colorectal cancer incidence and mortality: 20-year follow-up of five randomised trials. *Lancet*, *376*, 1741–1750. https://doi.org/10.1016/S0140-6736(10)61543-7

Sacco, A., Bruno, A., Contursi, A., Dovizio, M., Tacconelli, S., Ricciotti, E., Guillem-Llobat, P., Salvatore, T., di Francesco, L., Fullone, R., Ballerini, P., Arena, V., Alberti, S., Liu, G., Gong, Y., Sgambato, A., Patrono, C., FitzGerald, G. A., Yu, Y., & Patrignani, P. (2019). Platelet-specific deletion of cyclooxygenase-1 ameliorates dextran sulfate sodium-induced colitis in mice. *The Journal of Pharmacology and*

Experimental Therapeutics, *370*, 416–426. https://doi.org/10.1124/jpet.119.259382

Santilli, F., Rocca, B., de Cristofaro, R., Lattanzio, S., Pietrangelo, L., Habib, A., Pettinella, C., Recchiuti, A., Ferrante, E., Ciabattoni, G., Davì, G., & Patrono, C. (2009). Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays. *Journal of the American College of Cardiology*, *53*, 667–677. https://doi.org/10.1016/j.jacc.2008.10.047

Smith, J. B., & Willis, A. L. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. *Nature: New Biology*, *231*, 235–237. https://doi.org/10.1038/newbio231235a0

Taylor, D., Barnett, H., Haynes, R., Ferguson, G. G., Sackett, D. L., Thorpe, K. E., Simard, D., Silver, F. L., Hachinski, V., Clagett, G. P., Barnes, R., & Spence, J. D. (1999). Low-dose and high-dose acetylsalicylic acid for patients undergoing carotid endarterectomy: A randomized controlled trial; ASA and Carotid Endarterectomy (ACE) Trial Collaborators. ASA and Carotid Endarterectomy (ACE) Trial Collaborators. *Lancet*, *353*, 2179–2184. https://doi.org/10.1016/s0140-6736(99)05388-x

The Aspirin Myocardial Infarction Study Research Group. (1980). The aspirin myocardial infarction study: Final results. *Circulation*, *62*, v79–v84.

The CURRENT-OASIS Investigators. (2010). Dose comparisons of clopidogrel and aspirin in acute coronary syndromes. *The New England Journal of Medicine*, *363*, 930–942. https://doi.org/10.1056/NEJMoa0909475

The Dutch TIA Trial Study Group. (1991). A comparison of two doses of aspirin (30 mg vs. 283 mg a day) in patients after a transient ischemic attack or minor ischemic stroke. *The New England Journal of Medicine*, *325*, 1261–1266. https://doi.org/10.1056/NEJM199110313251801

The RISC Group. (1990). Risk of myocardial infarction and death during treatment with low dose aspirin and intravenous heparin in men with unstable coronary artery disease. *Lancet*, *336*, 827–830. https://doi.org/10.1016/0140-6736(90)92336-G

The SALT Collaborative Group. (1991). Swedish Aspirin Low-Dose Trial (SALT) of 75 mg aspirin as secondary prophylaxis after cerebrovascular ischaemic events. *Lancet*, *338*, 1345–1349. https://doi.org/10.1016/0140-6736(91)92233-R

Thun, M. J., Henly, S. J., & Patrono, C. (2002). Nonsteroidal antiinflammatory drugs as anticancer agents: Mechanistic, pharmacological and clinical issues. *Journal of the National Cancer Institute*, *94*, 252–262. https://doi.org/10.1093/jnci/94.4.252

Vane, J. R. (1964). The use of isolated organs for detecting active substances in the circulating blood. *British Journal of Pharmacology and Chemotherapy*, *23*, 360–373. https://doi.org/10.1111/j.1476-5381.1964.tb01592.x

Vane, J. R. (1969). The release and fate of vaso-active hormones in the circulation. *British Journal of Pharmacology*, *35*, 209–242. https://doi.org/10.1111/j.1476-5381.1969.tb07982.x

Vane, J. R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. *Nature: New Biology*, *231*, 232–235. https://doi.org/10.1038/newbio231232a0

Weiss, H. J., Aledort, L. M., & Kochwa, S. (1968). The effect of salicylates on the hemostatic properties of platelets in man. *The Journal of Clinical Investigation*, *47*, 2169–2180. https://doi.org/10.1172/JCI105903

Weksler, B. B., Pett, S. B., Alonso, D., Richter, R. C., Stelzer, P., Subramanian, V., Tack-Goldman, K., & Gay, W. A. Jr. (1983). Differential inhibition by aspirin of vascular and platelet prostaglandin synthesis in atherosclerotic patients. *The New England Journal of Medicine*, *308*, 800–805. https://doi.org/10.1056/NEJM198304073081402

Zucker, M. B., & Peterson, J. (1968). Inhibition of adenosine diphosphate-induced secondary aggregation and other platelet functions by acetylsalicylic acid ingestion. *Proceedings of the Society for Experimental Biology and Medicine*, *127*, 547–551. https://doi.org/10.3181/00379727-127-32737

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