REVIEW ARTICLE

Acetylsalicylic Acid (Aspirin): Past, Present, and Future

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Abstract

Acetylsalicylic acid is the most common antithrombotic drug, which started its pharmacological journey as a non-steroidal, anti-inflammatory drug. When used as a low-dose drug (of 75-100mg) once per day, it irreversibly inhibits prostaglandin H synthase, commonly termed cyclooxygenase 1 or COX-1 enzyme, which is acetylsalicylic acid's molecular drug target in human platelets. This mechanism of action ensures that the inhibition of the pro-aggregatory prostanoid - thromboxane A_2 synthesis is achieved permanently in platelets throughout their lifespan, which is responsible for acetylsalicylic acid's antithrombotic effect. In this literature review, we provide an overview of acetylsalicylic acid's development through history, the current understanding of the molecular mechanism of its action, as well as the resulting side effects impacting different tissues due to its control of the arachidonic acid metabolism and prostanoid synthesis in them. In an effort to begin a dialogue regarding the evidence in favor of unresponsiveness to acetylsalicylic acid's therapeutic effect in specific patients, we describe already identified molecular mechanisms of resistance to acetylsalicylic acid and list the existing biomarkers which are able to quantifiably measure the achieved degree of acetylsalicylic acid's clinical efficacy. Furthermore, we look to the future by encouraging a personalized approach to acetylsalicylic acid's use in order to maximize its therapeutic effect and its safety. Moreover, we mention the ongoing clinical trials evaluating the role of acetylsalicylic acid in prevention of colorectal and other cancers.

Keywords: Acetylsalicylic Acid, Aspirin, Cyclooxygenase Inhibitors, Prostaglandin Endoperoxide Synthase Inhibitors, Platelet Aggregation Inhibitors, Antithrombotic Agents

Introduction

Many pharmacological agents used in everyday clinical practice possess rich history, which is, firstly, associated with a plant or an animal source of their starting chemical compound and initial empirical use of it in patients. If deemed important for the treatment of patients, examinations of their molecular mechanisms and identification of their specific, molecular drug targets have taken place. Afterwards, pharmacokinetic/pharmacodynamic (PK/PD) studies would have been designed, performed and evaluated before their widespread clinical use. For many older pharmacological agents, different investigations during their drug development process have not followed this particular order, with PK/PD studies and large clinical trials being carried out without prior knowledge of drugs' molecular mechanisms, or their specific molecular drug targets. One such pharmacological agent is acetylsalicylic acid, which has become a household name through a widespread prescribing of its specific brand - Aspirin or aspirin - worldwide.

However, in 2024, acetylsalicylic acid still captures the imagination of clinicians and molecular pharmacologists alike. This interest is, in part, historical, for the purpose of teaching the next generations of medical doctors, pharmacologists and pharmacists about a timeline of the drug discovery and development process related to it. The current interest in acetylsalicylic acid is, however, largely clinical, as efforts are made towards identification of biomarkers for the quantifiable confirmation of the expected PD effect and more personalized prescribing of the low-dose acetylsalicylic acid in patients. In addition, as clinical trials evaluating efficacy of acetylsalicylic acid in the prevention of different cancers are coming to an end, acetylsalicylic acid could be entering into repurposed drug arena, all for the benefit of different patient populations.

History of acetylsalicylic acid development

The history of acetylsalicylic acid appears to have begun with the use of willow tree (of the genus *Salix*, family *Salicaceae*) for healing purposes in Mesopotamia (currently, parts of Iraq, Kuwait and Syria) several thousands of years ago, which was recorded on clay tablets in cuneiform writing (1). It also appears that a Greek physician and philosopher, the *"Father of Medicine"* - Hippocrates, had recommended the use of willow leaves for analgesia during childbirth and for the treatment of post-partum fever (2). The first, existing, written document in the English language, which describes in detail the preparation and the administration of the powdered bark of a white willow to patients suffering from *"agues and intermitting disorders"*, which were usually associated with malarial symptoms of fever and shivering, was a letter written by Reverend Edward Stone to the then president of the Royal Society in 1763 (3,4). In it, Reverend Stone stated the five year-long successful use of the described medicine towards the reduction of fever in 50 patients, *"except in a few autumual and quartan agues, with which the patients had been long and severely afflicted"*.

In the 19th century, the active ingredient of a willow bark, salicin, was extracted by several scientists (Figure 1). In addition, several scientists generated salicylic acid, either from salicin or by *de novo* chemical synthesis (Figure 1). Then, the first clinical trials using sodium salicylate or salicin took place in Germany, United Kingdom and France (5-8). In the United Kingdom, salicin was trialed for the treatment of *"acute articular rheumatism"*, which was accompanied with fever, by Dr Thomas John MacLagan, MD, in a Scottish town - Dundee, from 1874 until 1876 (7,9). Dr MacLagan's rationale for using salicin for the treatment of an acute rheumatic fever was that, if an extract of a bark of another tree, that of the cinchona tree, which is quinine, was successful in the treatment of a malarial fever, the same successful treatment could be achieved with salicin, as it is isolated from a bark of a tree found in an area where rheumatism is a prevalent disease. Reverend Stone and Dr MacLagan shared an opinion which was that the nature provides the remedies close to the areas of the disease. However, although both acute rheumatism and willow trees are predominating in damp climate, it was incorrectly assumed by both of them that cinchona tree originally grew in a malarious area, such as India, instead of in a non-malarious area, such as the Andes Mountains in South America. Dr MacLagan reported that the clinical effect of salicin, i.e., reduction in fever, was noticed 48 hours after the first administration of it, which led to his conclusion that the effect was specific to salicin (7,9).

After the publication of the positive results of the clinical trials performed by Stricker, Reiss, MacLagan and See, and development of the method for generation of synthetic salicylic acid on an industrial scale, the therapeutic application of it to achieve antipyretic, anti-rheumatic/anti-inflammatory and analgesic effects, to populations in many different countries, has started (10). During this time of worldwide administration of salicylic acid,

or its sodium salt - sodium salicylate, statements about its unpleasant taste and irritation to the gastrointestinal tract, accompanied with nausea and vomiting, have begun to emerge. In addition, patients who were prescribed high doses of either salicylic acid, or sodium salicylate, for the treatment of chronic rheumatic pain, experienced another unpleasant side effect - tinnitus (10). For these reasons, scientists started to consider developing a new compound, one which would retain the therapeutic effects of salicylic acid, but one which would be more palatable and devoid of the harmful side effects of the precursor compound. This task was given to a scientist employed at Bayer pharmaceutical company, Felix Hoffmann, in 1897. He successfully synthesized acetylated salicylic acid - acetylsalicylic acid, in chemically stable and pure form, on the 10th of August 1897, a date which is considered a birthday of acetylsalicylic acid. Bayer's trade name for acetylsalicylic acid - Aspirin, was registered in Berlin on the 1st of February 1899 and, soon after that date, widespread marketing and use of acetylsalicylic acid as Aspirin has begun (Figure 1) (10).

Mechanism of action of acetylsalicylic acid

In 1971, three seminal studies reported inhibition of prostaglandin production by acetylsalicylic acid and other non-steroidal, anti-inflammatory drugs (NSAIDs), such as indomethacin, including in human platelets,

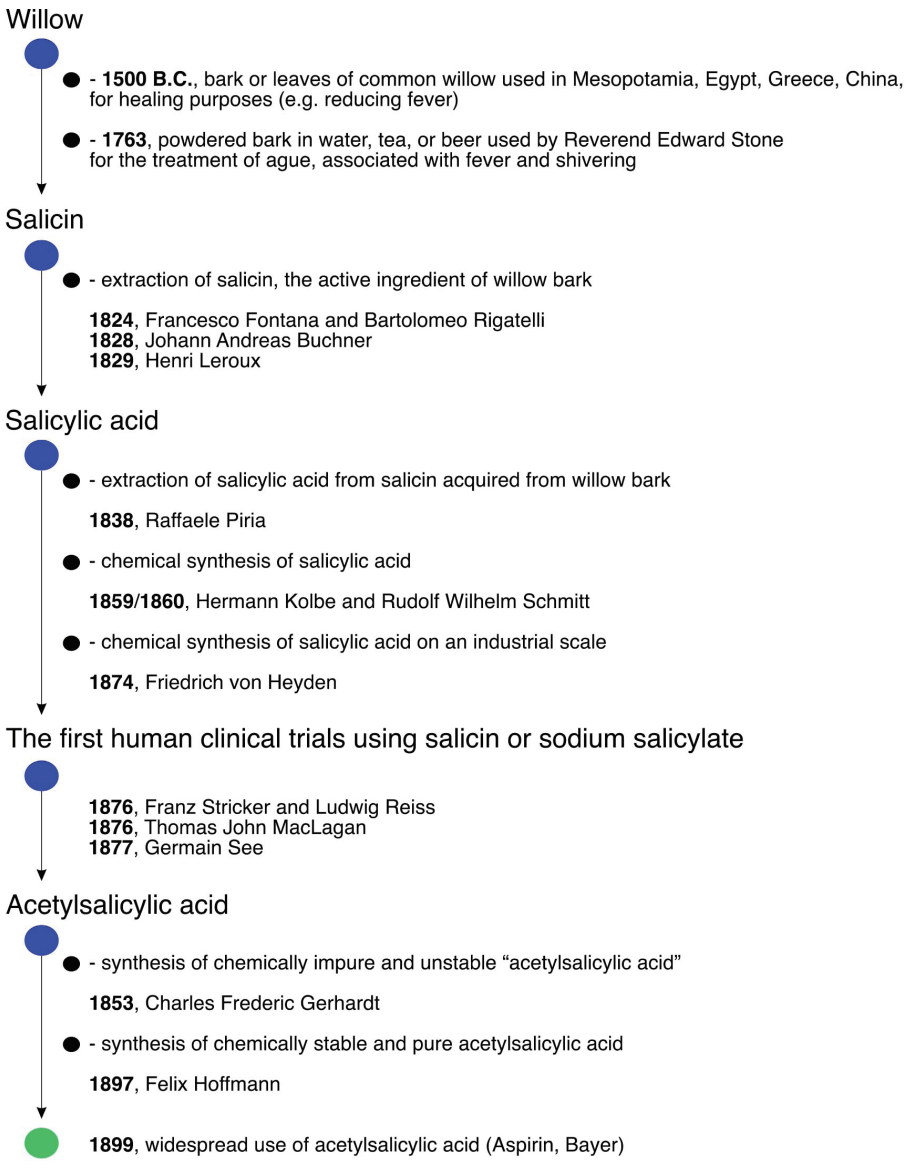


Figure 1. Historical development of acetylsalicylic acid

Development of acetylsalicylic acid is depicted as a list of key events which took place through history - from the use of bark or leaves of the common willow tree until the registration and widespread prescribing and use of acetylsalicylic acid as Aspirin by the pharmaceutical company - Bayer.

thereby describing, for the first time, the mechanism of action of acetylsalicylic acid (11-13). In addition, in 1975, another prostanoid - thromboxane (TX) A_2 (TXA_2), was identified as the major biologically active compound, which was responsible for induction of platelet aggregation. TXA_2 was shown to be derived from unstable prostaglandin endoperoxides, PGG_2 and PGH_2, respectively, generated from a fatty acid - arachidonic acid, by the enzyme prostaglandin H synthase (PGHS) (14). These studies established a mechanistic connection between the inhibition of the PGHS enzyme and inhibition of platelet aggregation by acetylsalicylic acid. In addition, it was demonstrated that acetylsalicylic acid acetylated the PGHS enzyme expressed in platelets, thus causing the inhibition of its activity (15). Furthermore, it was shown that arachidonic acid, the substrate for the PGHS enzyme, inhibited the enzyme's acetylation by acetylsalicylic acid, suggesting that acetylsalicylic acid acts at the active site of the PGHS enzyme (15). The enzyme PGHS, also known as prostaglandin endoperoxide synthase (PTGS), is a bifunctional enzyme, which incorporates two enzymes: cyclooxygenase and peroxidase. It is, at present, usually termed cyclooxygenase, or COX enzyme, even though this term leaves out the involvement of peroxidase activity in the enzyme's overall function (16). In summary, for acetylsalicylic acid, the mechanism of action was demonstrated approximately 85 years after the date of its marketing.

In humans, two cyclooxygenases, COX-1 and COX-2, are expressed (17). Although human COX-1 and COX-2 proteins share 61% identity in their amino acid sequences, there are specific differences between them, as follows: 1) the COX-1 and COX-2 proteins are encoded by two separate genes, and 2) the expression pattern of the COX-1 and COX-2 proteins, in the human organism, is different: while the COX-1 protein is continuously expressed in most tissues and cells, in a manner of a housekeeping protein, the COX-2 protein expression is largely considered to be inducible during development, or only after the release of pro-inflammatory cytokines, hormones, growth factors, etc. When the expression of the COX-2 gene/protein becomes induced, increased synthesis of the pro-inflammatory prostanoids occurs (18). The COX-1 protein/enzyme can become inducible protein/enzyme, e.g., upon increased release of endogenous bradykinin and, *vice versa*, the COX-2 protein/enzyme is a continuously expressed protein/enzyme, e.g., in the kidney (18). When acetylsalicylic acid is administered, it has the ability to acetylate both human COX enzymes. However, this occurs in a dose-dependent manner. In addition, its action is unique among NSAIDs as it covalently modifies the amino acid residues Ser-529 and Ser-516, localized within the active site of the human COX-1 and COX-2 proteins, respectively, causing an irreversible inhibition of the cyclooxygenase activities associated with both COXs (19).

The products of the COX-1 and COX-2 enzymes - prostanoids (prostaglandins and thromboxanes), mediate many physiological and pathological processes. Because of the fact that acetylsalicylic acid, as well as other NSAIDs, inhibit their generation and, consequently, limit their physiological function, knowing the expression pattern of the COX-1 and COX-2 proteins, in different organs and tissues of the human body, is important for our understanding of side effects which may develop when they are prescribed for the treatment of pathological states involving pain, fever and/or inflammation, especially for a prolonged period of time (Figure 2). Evidence suggests that both the COX-1 and COX-2 proteins are expressed in many human tissues albeit to varying degrees (20-21). Here, we list prostanoids which affect platelets, blood vessel wall, gastrointestinal tract, and kidney only, and describe effects of their reduced production, which develops upon the administration of acetylsalicylic acid, as they appear to have a significant impact in a wide population of patients.

Prostanoids in the platelets

In platelets, the COX-1 enzyme is the main enzyme for the production of TXA_2, the key prostanoid which induces platelet activa-

tion and aggregation and vascular smooth muscle cell contraction. Therefore, inhibition of the COX-1 enzyme activity by acetylsalicylic acid inhibits the production of TXA_2 and the TXA_2-induced platelet activation and aggregation and vascular smooth muscle cell contraction. In addition, it was demonstrated that platelets also express the COX-2 protein, albeit at low levels, which is capable of being induced to produce significant TXA_2 levels, especially in patients undergoing coronary artery bypass grafting (16) (22-24).

Prostanoids in the vascular system

In blood vessels, both endothelial cells and smooth muscle cells express the COX-1 protein. However, studies suggest that the COX-2 protein is expressed by endothelial cells only. Overall, it has been suggested that, under normal, healthy conditions, the vascular tissue predominantly expresses the COX-1 protein. The endothelial cells generate prostaglandin I_2 (PGI_2), or prostacyclin, which has the opposite characteristics to those of TXA_2: it inhibits the vascular smooth muscle cell contraction and prevents platelet aggregation. In addition, prostaglandin E_2 (PGE_2) contributes to the vasodilatation of blood vessels. Therefore, the administration of acetylsalicylic acid inhibits the release of vasodilatory prostanoids, i.e., PGI_2=prostacyclin and PGE_2, as well as diminishes anti-aggregation effect of PGI_2=prostacyclin on platelets (16).

Prostanoids in the gastrointestinal tract

In the gastrointestinal tract, studies suggest that both the COX-1 and COX-2 enzyme activities lead to the generation of PGE_2, especially in gastric mucosa, where PGE_2 plays a cytoprotective role against a mucosal damage by gastric acid. In addition, the release of appropriate level of PGI_2=prostacyclin maintains optimal blood flow within the gastrointestinal tract, which provides the energy for enabling stability of the gastrointestinal mucosa. Therefore, the most prominent side effect of acetylsalicylic acid, or other NSAIDS, especially in patients who have been prescribed a longer-term pharmacological therapy with these drugs, is the development of gastrointestinal injury, which is revealed by symptoms such as dyspepsia, abdominal pain, nausea, vomiting, mucosal lesions, peptic ulcers and/or gastrointestinal bleeding (16).

Prostanoids in the kidney

In the kidney, both the COX-1 and COX-2 proteins are expressed continuously under physiological conditions, i.e., without the induction by the pro-inflammatory stimuli. With regard to the expression of the COX-2 protein in the kidney in particular, it was suggested that the COX-2 expression is continuously induced by the shear stress of normal blood flow on endothelial cells of the renal arteries, rather than being constitutively expressed (16). Prostaglandins, PGE_2 and PGI_2=prostacyclin, which are generated through the activity of the COX-1 and COX-2 enzymes in the kidney, respectively, act as vasodilators, which increase renal blood flow. They also regulate sodium reabsorption, blood volume and, as a consequence, maintain blood pressure homeostasis (16,21).

In summary, different prostanoids, which act through their specific, G protein-coupled receptors specifically affect different cells and tissues. As the human body is not compartmentalized, when acetylsalicylic acid is administered, depending on its dose, all of the above cells and tissues could be affected by its mechanism of action simultaneously. Following prolonged administration of it, the resulting reduction in specific, vital prostanoids could lead to side effects or toxicity.

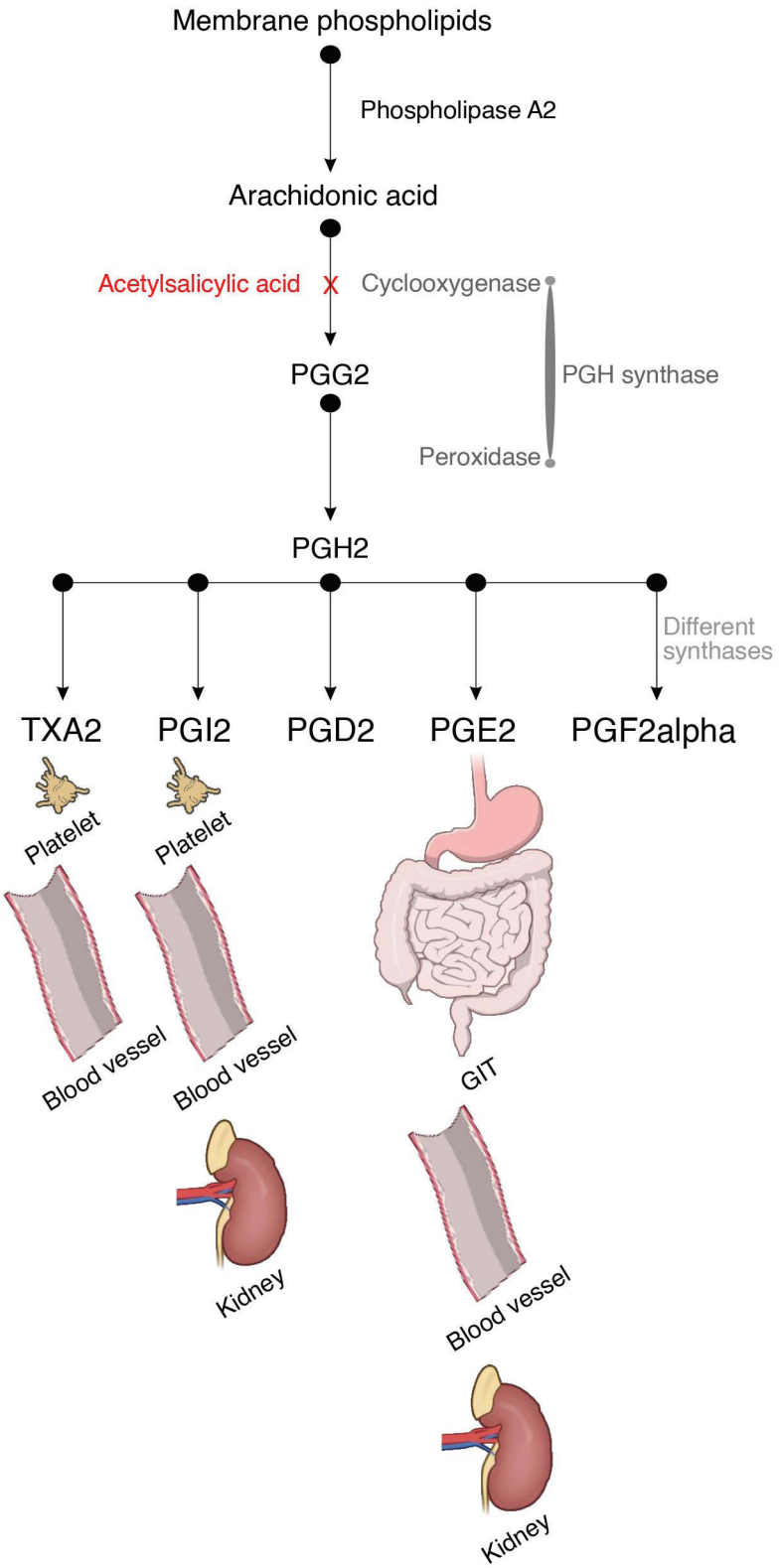
Current therapeutic use of acetylsalicylic acid as an antithrombotic drug

In 2024, acetylsalicylic acid is mainly prescribed as an antithrombotic drug. As platelets are the ultimate functional target of acetylsalicylic acid for the prevention of thrombosis, acetylsalicylic acid is often

referred to as an antiplatelet drug. Clinical trials, which started in the 1980s, were crucial in the evidence-based choice of the currently prescribed low-dose acetylsalicylic acid once per day (QD) for the prevention of cardiovascular disease (CVD) events (25-28). Doses of acetylsalicylic acid from 30-160mg/day were trialed and demonstrated effectiveness for the following CVD events: hypertension, stable angina, unstable angina, acute myocardial infarction, transient ischemic attack (TIA) and ischemic stroke,

Figure 2. Mechanism of action of acetylsalicylic acid

Acetylsalicylic acid inhibits the PGH synthase, currently termed cyclooxygenase or COX enzyme, which is its molecular drug target. When acetylsalicylic acid irreversibly acetylates the PGH synthase, the synthesis of physiologically active prostanoids, such as TXA2, PGI2, PGD2, PGE2 and PGF2alpha, is inhibited. This has implications for physiological function of different cells and tissues [e.g. platelets, blood vessels, kidney and gastrointestinal tract (GIT)]. The figure was created using illustrations from the National Institute of Allergy and Infectious Diseases, the National Institute of Health (NIAID NIH) BIOART Source: https://bioart.niaid.nih.gov/
X = inhibition; PGG2 = prostaglandin G2; PGH2 = prostaglandin H2; PGH synthase = prostaglandin H synthase; TXA2 = thromboxane A2; PGI2 = prostaglandin I2 or prostacyclin; PGD2 = prostaglandin D2; PGE2 = prostaglandin E2; PGF2alpha = prostaglandin F2α; GIT = gastrointestinal tract.



severe carotid artery stenosis, acute ischemic stroke (29).

A chosen dose of acetylsalicylic acid for the prevention of thrombosis needed to be sufficient to completely inhibit the COX-1 enzyme activity in platelets, in order to inhibit the COX-1 enzyme-dependent production of pro-aggregatory and anti-vasodilatory prostanoid - TXA_2. At the same time, the chosen dose of acetylsalicylic acid was required to spare the production of anti-aggregatory and pro-vasodilatory prostanoid - PGI_2=prostacyclin, which is produced by vascular endothelial cells, through both the COX-1 and COX-2 enzyme activities, as well as by the kidney, through the physiological activity of the continuously expressed COX-2 enzyme. A chosen dosing regimen of low-dose acetylsalicylic acid for an antithrombotic effect achieves almost complete, irreversible inhibition of the COX-1 enzyme expressed in platelets throughout their lifespan, which is approximately 9 days, as well as inhibition of the COX-1 enzyme in newly formed platelets, which are replaced every 24 hours (29). Following cessation of acetylsalicylic acid therapy, the recovery of platelets' physiological, baseline COX-1 enzyme activity and the synthesis of the pro-aggregatory TXA_2 levels, measured indirectly, through detection of the stable metabolite of TXA_2 in serum - TXB_2, occurs over a period of several days, which is consistent with platelets' turnover and the synthesis of new COX-1 protein/enzyme in them (27,30).

A summary of the latest clinical practice guidelines detailing the use of low-dose acetylsalicylic acid for primary and secondary prevention of atherosclerotic CVD events is presented:

1. The guideline by the U.S. Preventive Services Task Force (USPSTF), on the use of acetylsalicylic acid for primary prevention of CVD events, states the following: a) For a population aged 40-59 years of age, an individualized approach for low-dose acetylsalicylic acid (81mg QD) can be considered if a subject has 10% or higher 10-year CVD risk. The major concern for the use of acetylsalicylic acid in this population is the risk of bleeding, i.e., gastrointestinal bleeding; b) For a population having ≥60 years of age, due to a high risk of hemorrhage in the gastrointestinal tract and intracranial bleeding, the USPSTF does not recommend the use of acetylsalicylic acid for primary prevention of CVD events. In addition, the latest guidelines published by the USPSTF, on the use of acetylsalicylic acid for secondary prevention of CVD events, recommends the use of acetylsalicylic acid (75-100mg QD) unless contraindicated, i.e., having a high risk of hemorrhage. The individuals who belong to this group of patients usually have a diagnosis of previous CVD events, such as stroke, or myocardial infarction (31).

2. The guidelines which were published by the European Society of Cardiology (ESC) do not recommend the use of acetylsalicylic acid for primary prevention of atherosclerotic cardiovascular disease (ASCVD) due to an increased risk of hemorrhage (32).

3. The American College of Cardiology (ACC) stated that a low-dose acetylsalicylic acid (75-100mg QD) should be considered for primary prevention of ASCVD in a population aged 40-70 years, who are at higher ASCVD risk, but do not have a risk of hemorrhage. Also, the ACC does not recommend the administration of low-dose acetylsalicylic acid (75-100mg QD) for primary prevention of ASCVD in a population having >70 years of age (33).

4. The American Heart Association (AHA) does not recommend the use of low-dose acetylsalicylic acid (75-100mg QD) for primary prevention of ASCVD among populations of any age who are at an increased risk of hemorrhage (33).

5. The American Diabetes Association (ADA) recommends the use of low-dose acetylsalicylic acid (75-162mg QD) in a population having >50 years of age with diabetes mellitus and increased ASCVD risk, but without a history of vascular diseases (34, 35). The antithrombotic dose of acetylsalicylic acid does not affect the physiological, COX-2 enzyme-

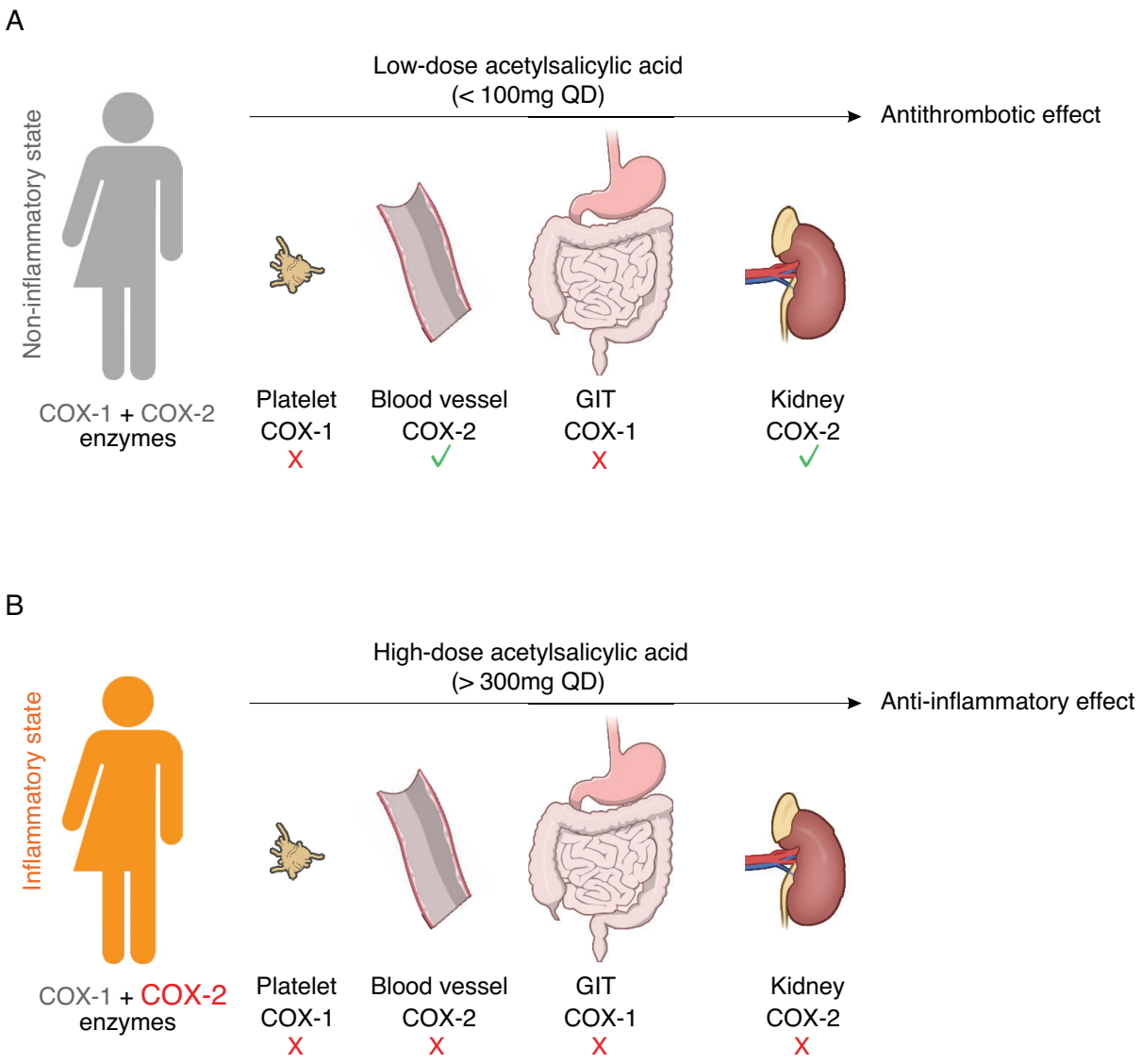


Figure 3. Pharmacological effects and side effects of low-dose versus high-dose acetylsalicylic acid

Current understanding of the pharmacological effects and cell- and tissue-specific side effects of low-dose versus high-dose acetylsalicylic acid, in individuals of both biological sexes, is presented.
A. A low-dose acetylsalicylic acid (of ≤ 100mg) is prescribed in order to achieve an antithrombotic effect. Patients who are prescribed a low-dose acetylsalicylic acid are in non-inflammatory state, which is characterised by physiological, constitutive expression of both the COX-1 and COX-2 enzymes. A low-dose acetylsalicylic acid inhibits the COX-1 enzyme in human platelets, thus inhibiting the synthesis of thromboxane A2 (TXA2), a prostanoid with a key function in platelet activation and aggregation. In addition, the COX-1 enzyme expressed in the gastrointestinal tract (GIT) is inhibited by a low-dose acetylsalicylic acid at least in part, with the resulting decrease in the levels of the prostaglandin - PGE2, causing the most common side effect of acetylsalicylic acid - a GIT upset or injury. Furthermore, as low-dose acetylsalicylic acid does not inhibit the COX-2 enzyme in blood vessels, the synthesis of the physiological levels of the PGI2=prostacyclin is maintained at an optimal level, thus providing optimal blood vessel dilatation and inhibition of platelet aggregation. Moreover, the constitutively expressed COX-2 enzyme in the kidney is also unaffected by low-dose acetylsalicylic acid, which contributes to the optimal vascular homeostasis in patients taking low-dose acetylsalicylic acid.
B. A high-dose acetylsalicylic acid (of >300mg) is prescribed in order to achieve an anti-inflammatory effect for the treatment of e.g. rheumatoid arthritis or osteoarthritis. Patients who are prescribed a high-dose acetylsalicylic acid or, either a different non-steroidal, anti-inflammatory drug (NSAID), or a COX-2 enzyme-specific inhibitor, are in an inflammatory state, which is characterised by pathological, induced expression of the COX-2 enzyme. A high-dose acetylsalicylic acid is required to inhibit the increasing levels of the COX-2 enzyme, which is 170-fold less sensitive to the inhibition by acetylsalicylic acid than COX-1 enzyme, and the resulting production of the pro-inflammatory prostanoids. As a consequence, the synthesis of physiologically active prostanoids is halted too. E.g. loss of the PGI2=prostacyclin synthesis leads to contraction of the vascular smooth muscle cells, platelet aggregation and kidney dysfunction, which clinically manifest as, e.g., pro-thrombotic and hypertensive states.
The figure was created using illustrations from the National Institute of Allergy and Infectious Diseases, the National Institute of Health (NIAID NIH) BIOART Source: https://bioart.niaid.nih.gov/

X = inhibition; ✓= intact activity; COX-1 (in black or gray color) = physiological, constitutive expression of the COX-1 enzyme; COX-2 (in black or gray color) = physiological, constitutive expression of the COX-2 enzyme; COX-2 (in red color) = induced expression of the COX-2 enzyme during an inflammatory state; GIT = gastrointestinal tract; QD = once per day.

dependent synthesis of PGI_2=prostacyclin, which takes place in tissues other than platelets, especially in the kidney. However, although the use of a higher dose of acetylsalicylic acid for the treatment of pain, fever and/or inflammation has largely been abandoned with the advent of new NSAIDs and COX-2 enzyme-specific inhibitors, it is important to remember that higher doses of acetylsalicylic acid (> 300mg) are capable of inhibiting both the COX-1 and COX-2 enzymes and, consequently, inhibit the systemic production of vasodilatory PGI_2=prostacyclin, as well as other prostanoids (27). As a result, higher doses of acetylsalicylic acid may give rise to gradual destruction of gastric mucosa barrier function, with formation of peptic ulcers, sometimes accompanied with gastrointestinal bleeding, as well as platelet aggregation, contraction of blood vessels, and kidney damage, resulting in dysregulated sodium and water homeostasis (Figure 3).

Future perspectives

The results of clinical trials using acetylsalicylic acid demonstrated that the degree of its effectiveness in the prevention of fatal and non-fatal vascular events depended on the clinical indication for which it was prescribed for, ranging from, e.g., approximately 15-50% (36). Therefore, a significant number of individuals are not protected with the drug, especially at the beginning of the treatment with it (37). This phenomenon was termed acetylsalicylic acid or aspirin resistance (38). It was demonstrated that it could develop as a result of several mechanisms, as follows: 1) changes in the expected PK characteristics of a patient; 2) presence of higher than expected platelet turnover and the resulting, significantly increased concentrations of pro-aggregatory TXA_2 in them, e.g., in patients with essential thrombocythemia; 3) induction of the COX-2 protein/enzyme expression in platelets, with consequent generation of substantial concentrations of TXA_2, e.g., in patients post coronary artery bypass grafting; 4) existence of genetic polymorphisms within genes encoding the COX enzymes expressed in platelets, or other type of, as yet undefined, protein alterations, rendering them resistant to interaction with acetylsalicylic acid; 5) overexpression of an efflux transporter protein, the multidrug resistance protein 4 (MRP4) in platelets, which was demonstrated to remove acetylsalicylic acid from cells, thus, reducing its effectiveness (23,24,39-44). In addition, studies showed that biological sex characteristics may play a role in different PK/PD effect of acetylsalicylic acid. Here, we mention the results of the placebo-controlled study by Ridker PM and colleagues, as it was a study of a significant size (about 40 thousand individuals were involved) and duration of follow up (about 10 years) (45). It was demonstrated that in individuals of female biological sex, who were ≥45 years of age and who took 100mg acetylsalicylic acid every second day for primary prevention of cardiovascular events for a minimum of 8 years, there was a statistically significant reduction in the risk of stroke. In particular, there was a reduction in ischemic stroke (while there was a non-significant increase in the risk of hemorrhagic stroke). In the treated females, there was no decrease in the risk of myocardial infarction. However, in the subgroup of females who were ≥65 years of age, the results showed a significant reduction in ischemic stroke and a reduction in myocardial infarction (45). In 2005, when the results of this study were published, the data for males indicated the opposite effect of acetylsalicylic acid, i.e., low-dose acetylsalicylic acid therapy was associated with a non-significant increase in the risk of stroke and a decrease in the risk of myocardial infarction (45).

The existing biomarkers of acetylsalicylic acid's overall pharmacological effect include serum TXB_2 and urinary 11-dehydro-thromboxane B_2 (11-dehydro-TXB_2), both physiologically inactive metabolites of physiologically active prostanoid - TXA_2. It was previously concluded that increased concentrations of urinary 11-dehydro-TXB_2 served as an indicator of an increased risk of cardiovascular events in patients to whom acetylsalicylic acid therapy was prescribed (46,47). Although the 11-dehydro-TXB_2 concentration measured in

urine does not appear to be a specific biomarker of an antithrombotic effect of acetylsalicylic acid, because it was demonstrated to be influenced by additional factors (e.g., increasing age, female sex, smoking, history of peripheral artery disease, oral hypoglycemic therapy, etc.), it does seem to serve as an indicator of the risk of stroke, myocardial infarction, and cardiovascular death in patients at risk of atherothrombotic events (47). Despite its disadvantages, urinary 11-dehydro-TXB_2 concentration will be measured as a biomarker of efficacy of acetylsalicylic acid in a clinical trial investigating the ability of acetylsalicylic acid to prevent the development of different cancers (48).

Since 1988, different studies have reported the existence of an association between regular use of acetylsalicylic acid and a reduced risk of colorectal cancer development (49). One of the key cellular mechanisms, which are responsible for colorectal cancer development and progression, includes activated platelets releasing pro-angiogenic and pro-inflammatory molecular factors, which interact with adjacent cells and drive the transformation of the normal colorectal mucosa to adenoma, carcinoma, and further on - to invasive and metastatic cancer cells, through enhanced COX-2 expression and the resulting synthesis of PGE_2. When acetylsalicylic acid is administered, PGE_2 levels are depleted through the inhibition of both COX enzymes in platelets. This, in turn, leads to increased apoptosis, decreased cellular proliferation and decreased angiogenesis, which are beneficial to cancer prevention (50). In order to establish if acetylsalicylic acid reduces the risk of development of multiple cancer types, a large placebo-controlled clinical trial titled ADD-ASPIRIN Trial was designed and approved in 2014. It will investigate if acetylsalicylic acid, of 100mg, or 300mg, affects colorectal, gastro-esophageal, breast and prostate cancers, with the primary outcome of the trial being disease-free survival, or overall survival, following a standard, primary therapy. The trial's results are expected to be published in 2025 and 2027 (48).

Overall, knowledge of the molecular mechanisms of acetylsalicylic acid's diminished pharmacological effect in specific patients, with the availability of established and emerging biomarkers, which are able to measure it precisely and quantitatively, allow for a design of studies to take place, which would establish a guideline for a personalized approach to acetylsalicylic acid's dose and dosing regimen in different patients, taking biological sex characteristics of patients into account. Considering the fact that the concentration of the main metabolite of TXA_2 synthesis, 11-dehydro-TXB_2, can be measured in an easily obtainable biological fluid - urine, it can be proposed that 11-dehydro-TXB_2 could be measured in patients in whom acetylsalicylic acid's resistance is suspected, unless very rapid effect is necessary, e.g., for the treatment of acute myocardial infarction within the first 24 hours following the onset of symptoms in patients. As it was established that almost complete inhibition of cyclooxygenase, the molecular target of acetylsalicylic acid, is achieved within 1 hour, following a single, 100mg dose of it, a delay in establishing the clinical response to acetylsalicylic acid in a patient appears minimal (30). Therefore, for a personalized approach to pharmacological therapy with acetylsalicylic acid, the existing tools allow fast determination of its initial efficacy, as well as its long-term monitoring. Alternatively, in the future, for patients who are unresponsive to acetylsalicylic acid, treatment with drugs which act at molecular targets downstream of the cyclooxygenases, COX-1 and COX-2, namely thromboxane synthase or thromboxane receptors, could become available for the inhibition of the production of, primarily, TXA_2, for a desired antithrombotic outcome (24,51).

Conclusion

This year marks the 125th anniversary of acetylsalicylic acid's registration and widespread use in different patient populations. Therefore, it is fitting that we are reminded

of the past, present, and future of acetylsalicylic acid this year. The ongoing investigations regarding the molecular mechanisms of resistance to acetylsalicylic acid's pharmacological effect, the clinical utility of the existing biomarkers, which allow measurement of its efficacy, and the clinical trials investigating the potential for re-purposing of acetylsalicylic acid towards prevention of different types of cancers, pave the way for the new era of acetylsalicylic acid's use, the one where no patient would be left behind without acetylsalicylic acid's therapeutic properties.

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