Review Article

The mechanism of action of aspirin

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Abstract

The therapy of rheumatism began thousands of years ago with the use of decoctions or extracts of herbs or plants such as willow bark or leaves, most of which turned out to contain salicylates. Following the advent of synthetic salicylate, Felix Hoffman, working at the Bayer company in Germany, made the acetylated form of salicylic acid in 1897. This drug was named "Aspirin" and became the most widely used medicine of all time. In 1971, Vane discovered the mechanism by which aspirin exerts its anti-inflammatory, analgesic and antipyretic actions. He proved that aspirin and other non-steroid anti-inflammatory drugs (NSAIDs) inhibit the activity of the enzyme now called cyclooxygenase (COX) which leads to the formation of prostaglandins (PGs) that cause inflammation, swelling, pain and fever. However, by inhibiting this key enzyme in PG synthesis, the aspirin-like drugs also prevented the production of physiologically important PGs which protect the stomach mucosa from damage by hydrochloric acid, maintain kidney function and aggregate platelets when required. This conclusion provided a unifying explanation for the therapeutic actions and shared side effects of the aspirin-like drugs. Twenty years later, with the discovery of a second COX gene, it became clear that there are two isoforms of the COX enzyme. The constitutive isoform, COX-1, supports the beneficial homeostatic functions, whereas the inducible isoform, COX-2, becomes upregulated by inflammatory mediators and its products cause many of the symptoms of inflammatory diseases such as rheumatoid and osteoarthritis.
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Aspirin is the most widely used drug in the world. An aspirin a day doubles the chances of a long life. Studies have shown that a regular dose of aspirin for the over 50s can prolong life since aspirin reduces the risk of many diseases associated with aging. The history of aspirin goes back many thousands of years to the early uses of decoctions or preparations of plants that contain salicylate. Maclagan [1] used Salicin, the bitter principle of the common white willow, successfully in 1874 to reduce the fever, pain and inflammation of rheumatic fever. Also in 1874, the commercial organic synthesis of salicylic acid was formulated by Kolbe and his colleagues and led to the founding of the Heyden Chemical Company.

The success of salicylic acid prompted the pharmaceutical manufacturing house of Frederick Bayer to actively search for a derivative of comparable or better efficacy to salicylic acid. Arthur Eichengrün, head of the chemical research laboratories at Bayer in 1895, assigned this task to a young chemist named Felix Hoffman. Hoffman also had personal reasons for wanting a more acceptable salicylic acid derivative; his father had been taking salicylic acid for many years to treat his arthritis and had recently discovered that he could no longer take the drug without vomiting. Impelled then by filial affection as well as by dedication to his job, Hoffman searched through the scientific literature and found a way of acetylating the hydroxyl group on the benzene ring of salicylic acid to form acetylsalicylic acid. After initial laboratory tests, Hoffman's father was given the drug; it was pronounced effective and later confirmed as such by a more impartial clinical trial.

The name "Aspirin" was given to the new drug by Bayer's chief pharmacologist, Heinrich Dreser [2], who was anxious to find a name that could not possibly be confused with salicylic acid. At least two accounts are given for Dreser's choice of name; some authorities maintain that the drug was named after St Aspirinius, an early Neapolitan bishop who was the patron saint against headaches. A more prosaic explanation is that the name was derived from *Spiraea*, which is the Linnaean name for the genus of plants to which meadowsweet belongs. Meadowsweet contains salicylaldehyde, which can be oxidised to salicylic acid. According to this explanation, the acid derived from *Spiraea* became "Spirsaüre" in German. Acetylation of Spirsaüre produced "Acetylspisaüre", which was soon shortened to Aspirin.

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Of course, it is quite possible that Dreser was aware of both possible derivations and that the ambiguous name was a deliberate and felicitous contrivance. Of interest, "Eûsparin" was suggested in the original document as an alternative name.

1. Early explanations for the action of aspirin

Before 1971, little was known about the real mechanism of action of aspirin-like drugs. They produced an anti-inflammatory effect that was qualitatively and quantitatively different from that of the anti-inflammatory steroids, and their analgesic action was of a different nature than that produced by opiates. Aspirin-like drugs are weak analgesics compared with "strong" narcotic analgesics like morphine. They are effective in clinical pain of low or moderate but not high intensity such as postoperative pain, osteoarthritis, rheumatoid arthritis, ankylosing spondilytis and some forms of headache [3]. Aspirin-like drugs are effective in experimental models involving the induction of a previous inflammatory state and block the delayed stretching response induced with an intraperitoneal injection of phenylbenzoquinone or dilute acetic acid in mice. They are not effective against nociception of short duration induced by pinching or stimulating the tail or toes of mouse, rat or guinea pig.

Guzman et al. [4] and Lim et al. [5] provided definitive evidence of the peripheral analgesic activity of aspirin-like drugs. What then does aspirin do in the periphery to decrease nociception or pain? Many biochemical effects of aspirin-like drugs have been documented and theories based on these effects have been abandoned. It was observed, for example, that most of these drugs uncoupled oxidative phosphorylation and that several salicylates inhibited dehydrogenase enzymes, particularly those dependent on pyridine nucleotides. Some aminotransferases and decarboxylases were also inhibited, as were several key enzymes involved in protein and RNA biosynthesis. All of these inhibitory actions [6] were at some time invoked to explain the therapeutic actions of aspirin. A problem with most of these theories was that the concentration of the drug required for enzyme inhibition was in excess (sometimes greatly in excess) of the concentration typically found in the plasma after therapy, and there was invariably a lack of correlation between the ability of these drugs to inhibit particular enzymes and their activities as anti-inflammatory agents. Perhaps the most serious impediment of all to acceptance of any of the above ideas was that their proponents could not provide a convincing reason why inhibition of any of these enzymes should produce the anti-inflammatory, analgesic and antipyretic effects of aspirin.

2. Aspirin and the prostaglandin system

It was against this background of knowledge that the investigation of aspirin's action was taken over by prosta-glandin (PGs) researchers. Piper and Vane [7] used isolated lungs perfused with Krebs' solution from sensitised guinea pigs. The purpose was to detect substances released during the anaphylactic reaction, including histamine and SRS-A, both of which had been known for many years as possible mediators of anaphylaxis.

They used the technique of continuous bioassay with the cascade bioassay system developed by Vane [8] in the middle 1960s for use with blood or artificial salt solution. As expected, Piper and Vane found the release during anaphylaxis of histamine and SRS-A, but they also found some previously unreported substances: PGs, (mainly PGE_2 but some $PGF_{2\alpha}$) and another, very ephemeral substance that they called "rabbit aorta contracting substance" (RCS) based on the assay tissue that identified it. In the lung perfusate RCS had a half-life of about 2 min; even when cooled to a few degrees above freezing, it remained stable for no more than 20 min. It was identified in 1975 as thromboxane A_2 by Hamberg et al. [9].

It was RCS that provided the first clue to the relation between aspirin and the PGs. In the course of further experiments involving RCS, Piper and Vane discovered that in some preparations RCS was released by bradykinin. This suggested that aspirin's ability to minimise some effects of bradykinin might be due to its blocking of RCS release. This idea was confirmed when Piper and Vane [10] presented experimental evidence that the release of RCS from isolated guinea pig lungs during anaphylaxis was blocked by aspirin. These guinea pig lung experiments also indicated that whenever aspirin blocked RCS release, there was a smaller contraction of the tissues that assayed PGs and a self-evident reduction in PG output after aspirin.

The natural result of these experiments was to move the focus of Vane's attention from RCS toward PGs. "While I was writing a review paper over the weekend", he recalled, "including the results of some of these experiments, a thought occurred to me that perhaps should have been obvious earlier on. In all these experiments (and in those of many other workers), the 'release' of PGs must in fact amount to fresh synthesis of PGs. That is, PG output in these experiments, though very low, was still far higher than the tissues' initial content of the hormones. Evidently, then, the various stimuli, mechanical and chemical, which released PGs, were in fact 'turning on' the synthesis of these compounds. A logical corollary was that aspirin might well be blocking the synthesis of PGs."

Vane immediately tested this exciting idea on the following Monday morning. In the absence of ram seminal vesicles, from which the synthetase enzyme was usually obtained, he used the supernatant of a broken cell homogenate from guinea pig lung, the same kind of preparation in which Änggård and Samuelsson [11] had detected the generation of PGE_2 and $PGF_{2\alpha}$ in 1965. Aliquots of the supernatant were incubated with arachidonic acid and different concentrations of aspirin, indomethacin or sodium salicylate. $PGF_{2\alpha}$ generation was

estimated by bioassay after 30 min of incubation at 37 °C. There was a dose-dependent inhibition of PG formation by all three drugs, indomethacin being the most potent and sodium salicylate the least. Three other drugs, morphine (an opiate analgesic), hydrocortisone (a steroidal anti-inflammatory) and mepyramine (an antihistamine), had little or no effect.

Vane [12] published the results of these experiments in *Nature* in 1971. Two other reports in the same issue lent support to his findings and extended them considerably. Both studies originated from the same department, and by coincidence, one of these stemmed from an entirely independent line of investigation.

Smith and Willis [13] were investigating the effects of aspirin on platelet behaviour. Venous blood samples were obtained from three colleagues before and 1 h after taking 600 mg of aspirin orally. Platelets were isolated, washed and incubated with thrombin and the supernatant was tested for the presence of various substances including PGs. No consistent changes were seen in the release of any of the substances except the PGs, which were substantially inhibited after aspirin. Indomethacin also blocked PG release when taken orally or when added directly to the platelets in vitro.

The importance of this study lay in its demonstration that these drugs were active not only in guinea pig lungs in vitro but also in humans, in platelets and after oral administration. In other words, the aspirin effect was not restricted by species, tissue or route of administration. These conclusions derived support from the final type of experiment reported in that issue of *Nature*. Ferreira et al. [14] demonstrated that the aspirin-like drugs blocked PG release from the perfused, isolated dog spleen. In the same year, Collier and Flower [15] reported in Lancet that administration of aspirin inhibited human seminal PG production.

3. Correlation of anti-enzyme activity of aspirin with its therapeutic activity

The major importance of these findings was that they provided a simple explanation of the manner in which aspirin-like drugs exerted their therapeutic actions. When the reports were published in 1971, there was already evidence suggesting that PGE_1 was an extremely potent pyretic agent in several species [16] and that PGE_1 or PGE_2 mimicked the inflammatory response when injected intradermally. PGs had also been detected in inflammatory exudates [17], so there were grounds for speculating that PGs might be responsible, at least in part, for the genesis of fever or inflammation and that the aspirin-like drugs might owe their therapeutic activity to their ability to prevent PG biosynthesis. Certainly, as Flower et al. [18] pointed out, the concentrations of these drugs required to inhibit synthesis were within the plasma levels found during therapy, even when protein binding was taken into account.

4. Inhibition of cyclooxygenase

A homogeneous, enzymatically active cyclooxygenase (COX) or prostaglandin endoperoxide synthase (PGHS) was isolated in 1976 [19]. This membrane-bound hemoprotein and glycoprotein with a molecular weight of 72 kDa is found in greatest amounts in the endoplasmic reticulum of prostanoid-forming cells [20]. It exhibits COX activity that both cyclizes arachidonic acid and adds the 15-hydroperoxy group to form PGG_2. The hydroperoxy group of PGG_2 is reduced to the hydroxy group of PGH_2 by a peroxidase that uses a wide variety of compounds to provide the requisite pair of electrons. Both COX and hydroperoxidase activities are contained in the same dimeric protein molecule.

Aspirin selectively acetylates the hydroxyl group of one serine residue (Ser 530) located 70 amino acids from the C terminus of the enzyme [21]. Acetylation leads to irreversible COX inhibition; thus, a new enzyme must be synthesized before more prostanoids are produced. When the purified enzyme is acetylated, only the COX, not the hydroperoxidase, activity is inhibited. The stoichiometry of this reaction is 1:1, with one acetyl group transferred per enzyme monomer of this dimeric protein. At low concentrations, aspirin acetylates PGHS rapidly (within minutes) and selectively. At high concentrations, over longer time periods, aspirin will also non-specifically acetylate a variety of proteins and nucleic acids [22]. Acetylation of the enzyme by aspirin places a bulky substituent on the Ser 530 oxygen that inhibits binding of arachidonic acid [23].

5. Discovery of COX-2 and COX-3

By the late 1980s, several reports appeared that the synthesis of PGHS could be stimulated by growth factors, tumour promoters, interleukin-1 [24], lipopolysaccharide and tumour necrosis factor. Interleukin-1 exerted its effect during the transcriptional rather than during the translational phase of induced synthesis of PGHS [25]. Induction of PGHS gene expression by serum factors occurred after approximately 2 h in mouse 3T3 cells, in which PGs are essential for cell division. These reports culminated with the discovery by Dan Simmons [26] of a second, distinct COX gene which could be induced with mitogens, growth factors, tumour promoters and lipopolysaccharide, and the induction of which could be inhibited with glucocorticoids. This gene expresses COX-2 which elaborates PGs, mostly PGE_2, during inflammatory reactions in contrast to COX-1, which produces PGs involved in physiological processes such as protection of the stomach mucosa, platelet aggregation and kidney function.

There is 60% homology between the amino acid structures of COX-1 and COX-2 and aspirin binds to Ser 516 in the active site of COX-2 in the same way as it binds to Ser 530 in the active site of COX-1. However, the active site of COX-2 is slightly larger than the active site of COX-1, so

that arachidonic acid can still 'squeeze past' the aspirin molecule inactivating COX-2 and become converted to 15-*R*-HETE [27].

The small difference in size between the active sites of COX-1 and COX-2 has been exploited by pharmaceutical companies to develop selective COX-2 inhibitors, such as celecoxib [28], rofecoxib [29] and meloxicam [30], which reduce inflammation without damaging the stomach mucosa. One company has also produced nitroaspirin [31], which combines aspirin with a nitric oxide-releasing moiety. The nitric oxide liberated in the stomach protects the stomach mucosa from damage by gastric hydrochloric acid. As new anti-inflammatory drugs with fewer severe side effects than non-selective non-steroid anti-inflammatory drugs (NSAIDs) are developed, the use of aspirin for osteoarthritis and rheumatoid arthritis will decline. However, its use as a potent anti-thrombotic agent for the prevention of second heart attacks is likely to increase.

A recent report from Chandrasekharan et al. [32] describes a third cyclooxygenase (COX-3) selectively inhibited not only by paracetamol but also by low concentrations of some non-steroid anti-inflammatory drugs including aspirin. COX-3 is a variant of COX-1 which has retained intron-1 during translation and which is found in human tissues in a polyadenylated form. Selective inhibition of COX-3 will discover potent and valuable new drugs for controlling pain and fever.

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