Inflammation and the mechanism of action of anti-inflammatory drugs

JOHN VANE AND REGINA BOTTING

The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ, United Kingdom

ABSTRACT

Inflammation is caused by release of chemicals from tissues and migrating cells. Most strongly implicated are the prostaglandins (PGs), leukotrienes (LTs), histamine, bradykinin, and, more recently, platelet-activating factor (PAF) and interleukin-1. Evidence for their involvement comes from studies with competitive antagonists for their receptors and inhibitors of their synthesis. H_1 histamine antagonists are effective for hay fever and some skin allergies such as urticaria, which indicates the importance of histamine in these conditions. Symptoms of rheumatoid arthritis are alleviated by the aspirinlike anti-inflammatory drugs, which inhibit the cyclo-oxygenase enzyme and reduce synthesis of prostanoids. Corticosteroids prevent the formation of both PGs and LTs by causing the release of lipocortin, which by inhibition of phospholipase A_2 reduces arachidonic acid release. They suppress the inflammation of rheumatoid arthritis and asthma. Currently, high doses of nonsedating H_1 antihistamines and PAF antagonists are being tested for the treatment of allergic asthma. — VANE, J.; BOTTING, R. Inflammation and the mechanism of action of anti-inflammatory drugs. *FASEB J.* 1: 89–96; 1987.

Key Words: inflammation · aspirin · salicylate · eicosanoids · corticosteroids

INFLAMMATION HAS BEEN STUDIED IN an attempt to deal with it for thousands of years. Celsius (in 30 A.D.) described the four famous signs of inflammation (rubor, calor, dolor, and tumor, or redness, heat, pain, and swelling) and used extracts of willow leaves to relieve them. Throughout the Roman times of Pliny the Elder, Dioscorides, and Galen, the use of salicylate-containing plants was further developed. In Asia and China also, salicylate-containing plants were applied therapeutically. Throughout the Middle Ages further uses were found, as plasters to treat wounds and various other external and internal applications, including the treatment of menstruation and dysentery.

On June 2, 1763, the Reverend Edmund Stone of Chipping Norton in Oxfordshire read a report to the Royal Society on the use of willow bark in fever. He had accidentally tasted it and was surprised by its extraordinary bitterness, which reminded him of the taste of cinchona bark (containing quinine), then being used to treat malaria. He believed in the doctrine of signatures that stated that the cures for diseases are often found in the same location where the malady occurs. Because the "willow delights in a moist and wet soil, where agues chiefly abound," he gathered a pound of willow bark, dried it on a baker's oven for 3 months, and pulverized it. His greatest success was with doses of 1 dram, which he reported using in about 50 patients with safety and success.

He concluded his paper (1) by saying "I have no other motives for publishing this valuable specific, than that it may have a fair and full trial in all its variety of circumstances and situations, and that the world may reap the benefits occurring from it."

His wishes have certainly been realized; world production of aspirin is many thousands of tons a year, with an average consumption in a developed country of about 100 tablets per person per year. Without the discovery in recent years of a great many substitutes for aspirin and its variants, consumption would surely be much higher.

Salicylic acid was chemically synthesized in 1860 in Germany and its ready supply led to even more extended usage as an external antiseptic, as an antipyretic, and in the treatment of rheumatism.

The father of Felix Hoffman, a chemist working for Bayer, urged his son to make a more palatable form of salicylate to treat his severe rheumatism. Felix made aspirin and asked his father to try it. Bayer's Research Director, Dr. Heinrich Dreser, recognized that he had an important new drug on his hands and introduced it in 1899, at the same time writing a paper suggesting that aspirin was a convenient way of supplying the body with the active substance salicylate (2). He suggested, therefore, that aspirin was acting as a prodrug and the anti-inflammatory actions of aspirin were caused by liberation of salicylate. Many other aspirinlike drugs are now available—the nonsteroid anti-inflammatory drugs (NSAIDs). We also have the anti-inflammatory steroids, but none of these substances is a cure or stops the progression of arthritis or chronic inflammation.

Inflammation covers a host of pathophysiological events and means different things to different people—

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acute or chronic, organ-specific such as asthma, reversible or irreversible—but one thing is certain, there are many mediators available: amines such as histamine and 5-hydroxytryptamine, short peptides such as bradykinin, long peptides such as interleukin-1 (IL-1), lipids such as prostaglandins (PGs) and leukotrienes (LTs), enzymes released from migrating cells, complement, and so on. How can we determine the importance of each of these putative mediators? We can do so by eliminating the mediator by preventing its generation with enzyme inhibitors, or by preventing its pharmacological effects with a selective antagonist.

MEDIATORS OF INFLAMMATION

Inflammatory mediators are also released in allergic asthma, which is accompanied by inflammation of the airways with increased numbers of inflammatory cells accumulating in the alveolar submucosa. Release of mediators from these cells may be responsible for the airway hyperreactivity that is a feature of bronchial allergic asthma.

Histamine

The release of histamine from mast cells during antigen-antibody reactions is well known, as is its involvement in the inflammatory response to skin injury. Also, increased numbers of mast cells are present in the rheumatoid synovium and in the asthmatic lung, correlated with raised levels of histamine (3).

When the first antihistamines were discovered in the 1940's, it was hoped that they would be potent anti-inflammatory agents and, indeed, they found a role in the treatment of hay fever and some cutaneous inflammation. But these H_1 antihistamines are ineffective in arthritis or asthma, so that histamine did not seem to play a major part in these conditions.

The advent of nonsedating H_1 antihistamines has allowed them to be tested in much higher doses than ever before, and some evidence suggests that histamine may, after all, play a role in allergic asthma (4).

Bradykinin

Small amounts of bradykinin cause pain, vasodilatation, and edema, all contributing to inflammation. Bradykinin-like immunoreactivity has been detected in rat pleural inflammatory exudates. Kinins are also present in nasal secretions after immunological challenge, and a kininogenase is released from human lung mast cells (5). Inhaled bradykinin causes bronchoconstriction in normal and asthmatic individuals, but not through release of PGs (6). Lack of effective antagonists makes it difficult to assess the extent of involvement of kinins in inflammation and asthma, but there is no evidence that inhibitors of the inactivation of bradykinin, such as captopril or enalopril, exacerbate these conditions.

The prostaglandins

Apart from nonnucleated erythrocytes, all cells are capable of synthesizing PGs, which are released in response to many kinds of trauma or any disturbance of the cell membrane. In 1971 Vane discovered that aspirin and similar drugs inhibit the biosynthesis of PGs, and proposed that this explained their mechanism of action (7). In other words, the pathological release of PGs that contributes to inflammation, fever, and pain is inhibited by aspirin and other NSAIDs. The aspirinlike drugs also share, to a greater or lesser extent, certain side effects, such as a propensity to irritate the stomach, nephrotoxicity in high concentrations, and interference with the birth process. It was suggested that these side effects resulted from the inhibition of the physiological release of a protective PG.

Thromboxane A_2 and prostacyclin

The antiplatelet effects of aspirin could not be explained by inhibition of the synthesis of PGE_2 or $PGF_{2\alpha}$ because these PGs do not affect platelet aggregation to any great extent. However, in 1975 Samuelsson discovered that in platelets arachidonic acid (AA) is metabolized to the proaggregatory thromboxane (TX) A_2 (8). Aspirin was shown to inhibit the formation of the endoperoxide intermediate in this pathway (9) (Fig. 1). Soon after the discovery of TXA_2, another prostaglandin was discovered that showed opposite activity to that of TXA_2 (10). Prostacyclin, as it was later termed, relaxes blood vessels and inhibits aggregation of platelets. Its synthesis in endothelial cells of blood vessel walls is of special importance (11).

The leukotrienes

Slow-reacting substance of anaphylaxis (SRS-A) was identified as a product of the 5-lipoxygenase pathway of AA metabolism (12), and Samuelsson renamed the constituents of SRS-A as leukotrienes (LTs). In contrast to its inhibitory effects on cyclo-oxygenase, aspirin does not in-

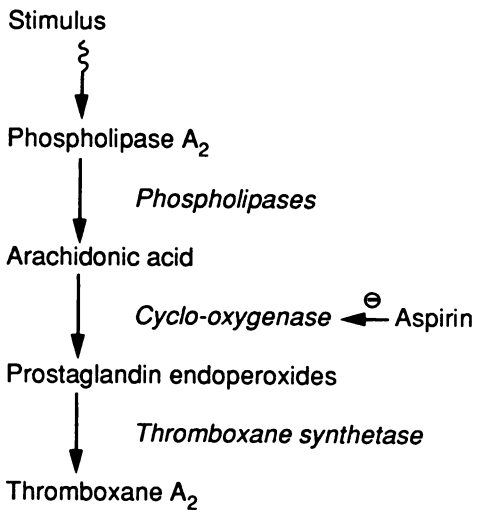

Figure 1. Action of aspirin on platelets.

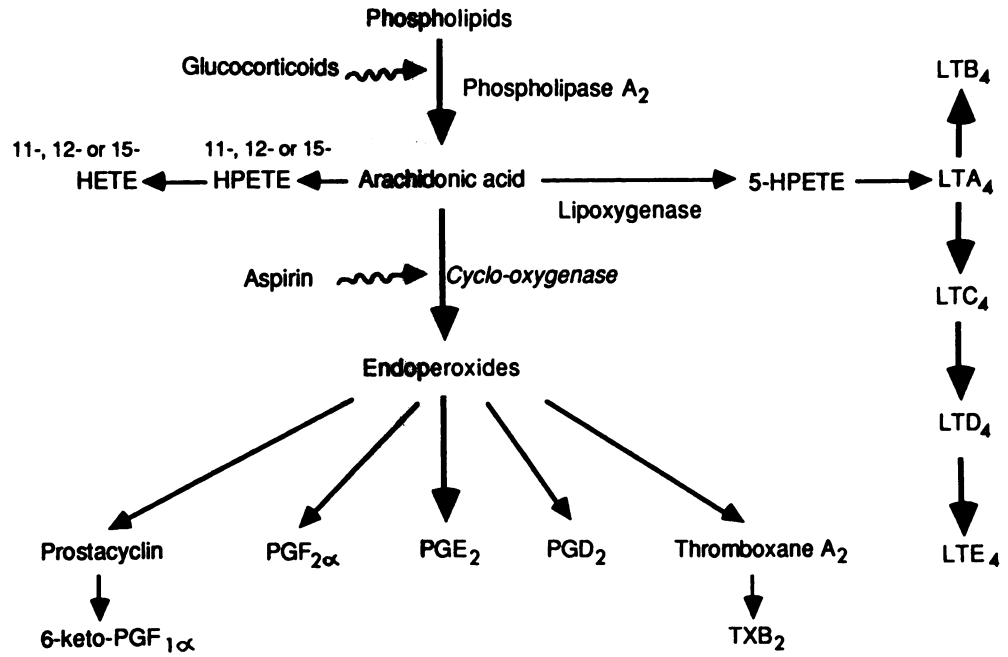

Figure 2. Catabolic pathways of AA.

hibit 5-lipoxygenase and, therefore, neither does it inhibit LT synthesis (Fig. 2). There is some evidence that lipoxygenase products contribute to vascular changes in inflammation. In the guinea pig skin and hamster cheek pouch, LTC_4 and LTD_4 potently increased permeability of venules to plasma macromolecules. LTB_4, LTC_4, and LTD_4 cause transient wheal and flare reactions in human skin, and LTE_4 is equipotent with histamine in increasing permeability of venules in the abdominal muscles of anesthetized guinea pigs (13). The LT antagonist FPL-55712 substantially inhibits LTC_4-induced bronchoconstriction and cough (14), but when administered to chronic asthmatics, FPL-55712 was only weakly active. FPL-55712 is now known to be a selective antagonist for the LTD_4 receptor, which could be related to its low activity in asthma. Antagonists at LTC_4, LTD_4, and LTE_4 receptors such as LY 171,883 may be more effective antiasthma drugs.

Platelet-activating factor (PAF)

The phospholipid PAF-acether is released by the action of phospholipase A_2 from most proinflammatory cells, as well as by vascular endothelial cells and platelets (15). It induces inflammatory reactions in various animal species and in human skin (16). PAF also mimics the main clinical features of asthma and is particularly effective in producing hyperreactivity and accumulation of eosinophils in lung tissue. Asthmatic patients have high levels of circulating PAF (17) and their eosinophils make more PAF than those of normal controls. The antiasthmatic glucocorticoids, by suppressing phospholipase A_2, will thus reduce the formation of PAF. Furthermore, PAF antagonists, such as the ginkgolides, are currently being investigated for the treatment of asthma.

Interleukin-1

IL-1 is a polypeptide produced by activated macrophages that mimics the symptoms of chronic inflammation (18). It has had other names, including endogenous pyrogen. IL-1-like activity (equivalent to 1.69 U/ml) has been detected in synovial fluids from patients with rheumatoid arthritis (19). Its actions include activation of lymphocytes and production of fever, the latter being mediated by release of PGE_2.

Intra-articular injections of highly purified IL-1 into rabbit knee joints caused swelling, accumulation of polymorphonuclear and mononuclear leukocytes, and the loss of proteoglycan from the articular cartilage. The inflammatory changes were similar to those seen in the joints of rabbits with antigen-induced arthritis 1–14 days after antigen challenge. IL-1 stimulates the release from cultured synovial cells and chondrocytes of collagenase that may be responsible for the cartilage breakdown (20).

MECHANISM OF ACTION OF NONSTEROID ANTI-INFLAMMATORY AGENTS

The concentration of a PGE_2-like substance is about 20 ng/ml in the synovial fluid of patients with rheumatoid arthritis. This decreases to zero in patients taking aspirin, demonstrating its effect on PG synthesis clinically (21). Carrageenan-impregnated polyester sponges implanted s.c. in rats were used to induce experimental inflammation (22). Periodic examination of the inflammatory exudate contained within the sponges showed that the concentration of PGE_2 increased throughout the 24-h experiment. In addition, the output of TXA_2 and LTB_4 increased to a peak after 4–6 h and then declined over the remainder of the experiment (Fig. 3). PGE_2 causes vasodilatation and hyperalgesia, and the

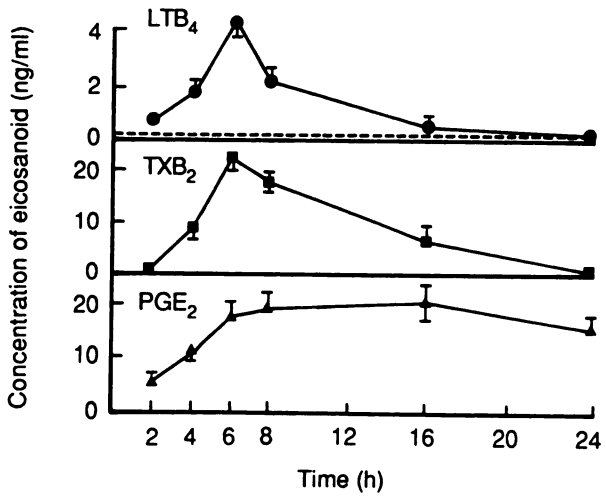

Figure 3. Concentrations of eicosanoids in inflammatory exudates with the minimum detectable concentration level (-----). From ref 22. Reprinted with permission from *Biochem. Pharmacol.*, vol. 32, Simmonds, P. M.; Salmon, J. A.; Moncada, S., The release of leukotriene B_4 during experimental inflammation, Copyright 1983, Pergamon Journals Ltd.

chemotactic property of LTB_4 probably attracts polymorphonuclear leukocytes into the region (23). However, the role of TXA_2 in the inflammatory response is not understood.

Evidence supporting the role of PGs in the inflammatory reaction was obtained by using carrageenan to induce inflammation in the rat paw. The release of endogenous PGs was eliminated by aspirin, and then administration of low doses of exogenous PGE_2 (1.0 ng) or prostacyclin (10 ng) caused an increase in edema (24).

The possibility that aspirinlike drugs influence the release of other substances, such as histamine and bradykinin, was experimentally discounted and further studies were designed to show that the antienzyme effect of aspirinlike drugs correlated with their anti-inflammatory effects. Comparing the effects of two optical isomers of naproxen, Tomlinson et al. (25) showed that the one that possesses anti-inflammatory properties (in adjuvant arthritis and carrageenan edema) was also a potent inhibitor of PGE_2 synthesis. The other isomer was much less active in all the tests. In a survey of a whole range of NSAIDs at therapeutic doses, the peak plasma concentrations, even allowing for protein binding, were more than sufficient to inhibit PG formation in an isolated enzyme preparation (26) (Table 1).

After demonstrating that the anti-inflammatory effects of NSAIDs are mediated via inhibition of PG synthesis, it was pertinent to determine whether a similar mechanism underlies the side effect profile of aspirin. This has been addressed with regard to the ulcerogenic potential of aspirin, and it is now known that prostacyclin is an important cytoprotective product of the gastric mucosa. Administration of various PGs reverses or prevents experimental gastric ulcers, and some of the recently developed PG derivatives are now available for clinical use. On the other hand, the antienzyme activity of several NSAIDs correlates with their capacity to erode the gastric mucosa. In the clinic, NSAIDs suppress mucosal PG formation. However, salicylate decreases PG concentration in inflammatory exudate without affecting production by the gastric mucosa, and it possesses a very low erosion index (27). It is not known why salicylate differs from other aspirinlike drugs in this manner.

THE MECHANISM OF ACTION OF STEROIDS IN INFLAMMATION

Corticosteroids inhibit phospholipase A_2 activity, which is necessary for the release of AA. Thus, corticosteroids ultimately inhibit the formation of PGs, TX, and the LTs. Anti-inflammatory steroids inhibit phospholipase A_2 indirectly by the release of an inhibitory protein. This has been variously termed macrocortin, lipomodulin, or renocortin, and molecular sizes of 15, 30, and 40 kDa have been reported. The name lipocortin has been agreed upon (28) and a pure, cloned form has recently become available and is claimed to be a potent anti-inflammatory agent (29).

There is now some dispute with respect to its mode of action, for lipocortins appear to be identical to calpactins. Calpactins bind calcium and also phospholipid, and it has been suggested that this property, rather than the direct inhibition of phospholipase A_2, is responsible for the reduction in eicosanoid formation (30).

TABLE 1. *Inhibition of PG formation by anti-inflammatory drugs*[a,b]

	Synthetase ID_{50}, μg/ml	Rat paw edema ED_{50}, mg/kg	Peak plasma concentrations in humans, μg/ml (% protein binding)
Meclofenamic acid	0.03	15	
Niflumic acid	0.03	47	100 (90%)
Indomethacin	0.06	6	2 (90%)
Mefenamic acid	0.17	68	10 (48%)
Phenylbutazone	2.23	100	150 (98%)
Alclofenac	3.3	~100	26 (10%)
Aspirin[c]	6.6	150	55 (80%)
Paracetamol	~100.0	Inactive	50 (25%)

[a] From ref 26. [b] Assay was done on a particulate enzyme preparation from dog spleen. [c] Note that the peak plasma concentration of aspirin is expressed as salicylate.

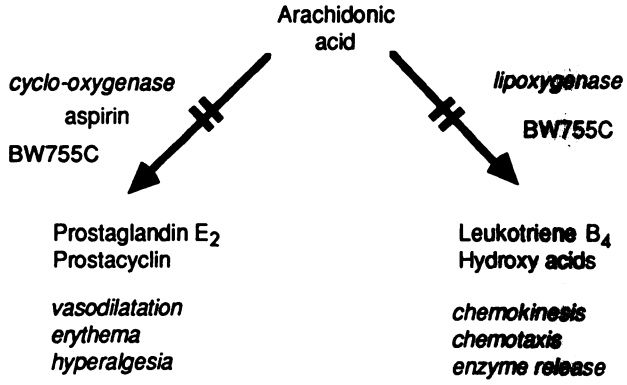

Figure 4. Dual activity of BW755C.

DUAL INHIBITION OF CYCLO-OXYGENASE AND 5-LIPOXYGENASE

By preventing AA release, steroids neutralize the two main pathways of the AA cascade and are powerful anti-inflammatory agents. Drugs that inhibit both cyclo-oxygenase and 5-lipoxygenase would be expected to possess the same anti-inflammatory activity as steroids but with the advantage of not containing a steroid nucleus. BW755C inhibits both enzymes (31) (Fig. 4), but it also causes hemolysis and will not be available for clinical use. In the sponge model of inflammation, equivalent anti-inflammatory doses of BW755C and dexamethasone similarly inhibited PG production and cell migration. However, indomethacin only suppressed PG production (Fig. 5).

As a result of these encouraging pharmacological effects, there are several other dual inhibitors of cyclo-oxygenase and 5-lipoxygenase currently undergoing development. It is anticipated that they may possess therapeutic effects superior to conventional aspirinlike drugs in chronic inflammation or anaphylactic bronchoconstriction.

Selective 5-lipoxygenase inhibitors are also undergoing clinical investigation. Piriprost, an inhibitor of LT formation, blocks LT release from lung tissue of asthmatic patients challenged with allergen. In vivo, piriprost inhibits bronchoconstriction in sensitized monkeys induced by inhalation of *Ascaris suum* antigen (32). However, piriprost administered to asthmatics failed to prevent the bronchoconstriction resulting from allergen challenge (33). It is possible that blocking of the lipoxygenase pathway may shunt AA toward increased formation of bronchoconstrictor cyclo-oxygenase products such as PGD_2.

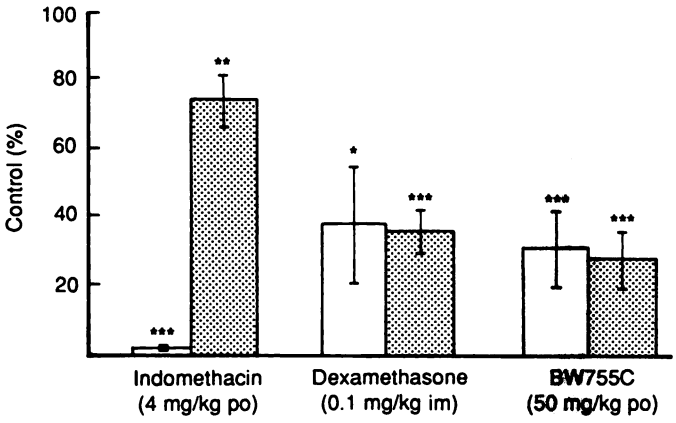

Figure 5. Inhibition of PG production (open bars) and leukocyte migration (stippled bars). $*P = < 0.1$. $**P = < 0.01$. $***P = < 0.005$. From ref 31. Reprinted with permission from *Biochem. Pharmacol.*, vol. 28, Higgs, G. A.; Flower, R. J.; Vane, J. R., A new approach to anti-inflammatory drugs, Copyright 1979, Pergamon Journals Ltd.

DIFFERENCES BETWEEN ASPIRIN AND SALICYLATE

Aspirin and salicylate are considered to be equally potent as anti-inflammatory agents, but salicylate is less potent than aspirin in inhibiting PG synthesis by a crude enzyme preparation from guinea pig lung (7). In contrast, an anti-inflammatory dose (3 g) of aspirin or salicylate reduced the urinary output of PG metabolites in humans by 85–95% (34).

When prostacyclin was discovered and its antithrombotic effect realized, clinical studies were already in progress to investigate whether aspirin possessed antithrombotic activity by inhibiting TXA_2 synthesis. Subsequently, the dose of administered aspirin was progressively reduced in the hope of selectively inhibiting TXA_2 production while preserving prostacyclin synthesis (35). This was achieved by administering 40 mg

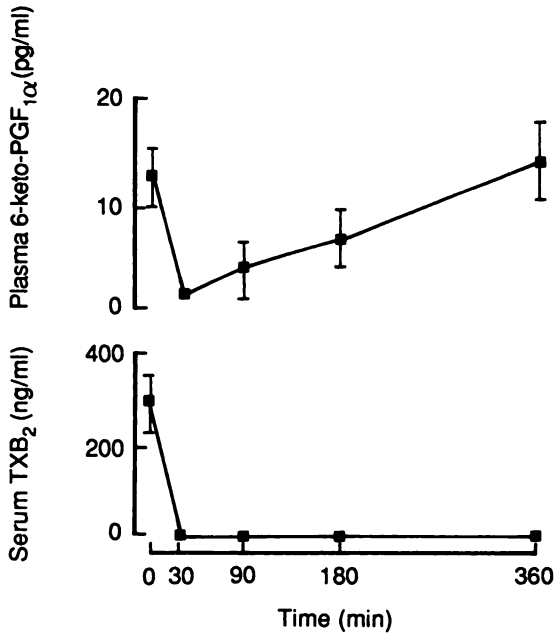

Figure 6. Effect of aspirin on serum TXB_2 concentration and bradykinin-stimulated prostacyclin production. Values shown are means ± SEM ($n = 7$). Plasma 6-keto-$PGF_{1\alpha}$ concentrations are those measured at the end of each bradykinin infusion. Blood (2 ml) taken immediately before each bradykinin infusion was assayed for TXB_2, the stable hydrolysis product of TXA_2. In contrast to the effects on bradykinin-stimulated prostacyclin production, serum TXB_2 remained more than 99% inhibited 360 min after 600 mg aspirin. Bradykinin infusion was performed at 0, 30, 90, 180, and 360 min. From ref 37. Reprinted by permission from *Nature*, Vol. 318, pp. 186–188, Copyright © 1985 Macmillan Journals Limited.

aspirin daily, and it led to a re-evaluation of the pharmacokinetics of aspirin and salicylate to explain this low-dose selectivity. In males who were given therapeutic doses of aspirin daily, the peak systemic plasma concentration of aspirin was about 2 μg/ml after about 15 min. Within 1–5 h the plasma aspirin concentration had decayed to zero. However, the peak plasma salicylate concentration, formed by deacetylation of aspirin, increased to about 10 times that of aspirin and was maintained for several hours. Aspirin irreversibly inhibits platelet cyclo-oxygenase. Therefore, it was proposed that after gastrointestinal absorption, aspirin inhibits platelet cyclo-oxygenase within the presystemic circulation. About 60% of the absorbed aspirin is deacetylated to salicylate during the first pass through the liver, and the resulting plasma aspirin concentration in the systemic circulation may well be too low to be associated with any significant cyclo-oxygenase-inhibiting activity in systemic tissues, including the vessel wall (36).

Another contributing factor to the selective inhibition of platelet TXA_2 production by aspirin is that platelets cannot regenerate cyclo-oxygenase. To stimulate prostacyclin production, infusions of bradykinin were given to volunteers (37) (Fig. 6). Two tablets of aspirin blocked TXA_2 production by platelets for the duration of the experiment, but prostacyclin production recovered within 6 h, which possibly reflects the capacity of cells other than platelets to regenerate cyclo-oxygenase (38).

Prompted by these findings, experiments were conducted to measure the concentrations of aspirin and salicylate in inflammatory exudate with the carrageenan-impregnated polyester sponge implants (39). The findings paralleled the previously described pharmacokinetic study in volunteers. After oral administration of aspirin (200 mg/kg) to these rats, the peak concentration of aspirin (about 1.5 μg/ml) in inflammatory exudate was considerably lower than that of salicylate (Fig. 7). Furthermore, administration of aspirin or salicylate equally reduced the concentrations of PGE_2 and TXB_2 in inflammatory exudate. Finally, the effects of aspirin and salicylate on the inhibition of cyclo-oxygenase in explants of inflamed tissue were examined (Table 2). Comparison of the potencies with the relative concentrations of aspirin and salicylate measured in inflammatory exudates after oral administration showed that, although aspirin did not reach high enough concentrations to inhibit cyclo-oxygenase to any great extent, there was a sufficient concentration of salicylate to considerably inhibit PG synthesis (Table 3).

Thus, as Dreser suggested in 1899, aspirin may be a prodrug to deliver salicylate to its site of action.

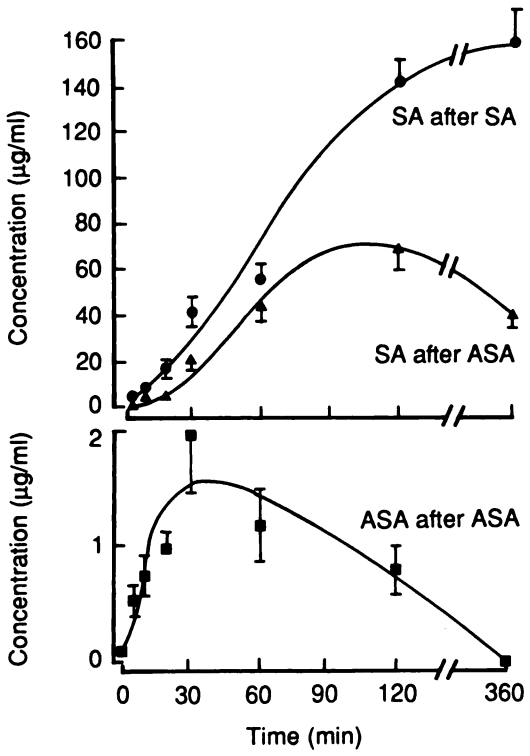

Figure 7. Concentration of salicylate (SA) and aspirin (ASA) in exudate after oral doses of 200 mg/kg. From ref 39.

TABLE 2. *Inhibition of cyclo-oxygenase in explants of inflamed tissue*[a]

	ED_{50}, μg/ml	ED_{75}, μg/ml
Aspirin	4.2	10.0
Salicylate	10.0	90.0

[a] From ref 39.

OTHER ANTI-INFLAMMATORY DRUGS

Gold

Gold salts modify the disease process in rheumatoid arthritis. Aurothiomalate and aurothioglucose are given by injection whereas auranofin is orally active. They suppress the function of the immune system by reducing the activity of macrophages (40) and lymphocytes. In addition, aurothiomalate reduces numbers of circulating lymphocytes, and auranofin inhibits the release of PGE_2 from synovial cells and the release of LTB_4 and LTC_4 from polymorphonuclear leukocytes (41).

Colchicine

The inflammatory response to urate crystals in gouty arthritis is dramatically abrogated by colchicine. Anti-microtubule agents such as colchicine reduce poly-

TABLE 3. *Concentration of aspirin (ASA) and salicylic acid (SA) in inflammatory exudates 0.5–6 h after oral administration of ASA or SA*[a]

	Concentrations, μg/ml					
	0.5 h		2 h		6 h	
Drug given	ASA	SA	ASA	SA	ASA	SA
ASA (200 mg/kg)	2	21	0.8	73	0	41
SA (200 mg/kg)	0	43	0	144	0	160

[a] From ref 39.

morphonuclear chemotaxis and lysosomal enzyme release. However, colchicine is unique in preventing the release of a glycoprotein (8400 Da) that is chemotactic for neutrophils and monocytes by polymorphonuclear leukocytes that have phagocytosed urate crystals. It does not prevent the inflammatory response to intra-articular injection of the purified glycoprotein (42).

CONCLUSION

In conclusion, we have learned much about the mediators of inflammation, and there are drugs available that inhibit the production or actions of some of these. However, there is no drug available that will stop the progression of arthritis or asthma effectively. Significant future developments in the treatment of inflammatory disease may arise from nontoxic dual inhibitors of the AA cascade. In addition, inhibitors of the endogenously produced PAF and of IL-1, which have also been associated with inflammatory disorders, may become the anti-inflammatory drugs of the future. FJ

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