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TARGETS FOR ANTIINFLAMMATORY DRUGS

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ABSTRACT

Aspirin-like nonsteroidal antiinflammatory agents, corticosteroids, and methotrexate are the mainstays of therapy in rheumatoid arthritis and other inflammatory diseases. It is now clear that these agents share at least one characteristic: All of these agents diminish the adhesive interactions required for the accumulation of white blood cells at an inflamed site. We discuss the biochemical and functional mechanisms by which these drugs alter the inflammatory response, and the potential for development of new antiinflammatory agents based upon these pharmacological actions.

INTRODUCTION

During the past fifteen years, our understanding of the mechanisms and molecules of inflammation has expanded dramatically. The discovery of entirely new classes of protein and lipid mediators of inflammation, the elucidation of the role of endothelial-leukocyte adhesion, and the isolation and characterization of the molecules that mediate adhesion have clearly changed our perception of the pathogenesis of inflammation. Along with providing a newer model of the pathogenesis of inflammation, we have changed our understanding of the mechanism of action of various antiinflammatory agents and have begun to take new approaches to the development of drugs for the therapy of such inflammatory diseases as rheumatoid arthritis. In this review we discuss the mechanism of action of three of the most commonly used antirheumatic agents:

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corticosteroids, nonsteroidal antiinflammatory agents (aspirin-like drugs), and methotrexate. Although these three agents inhibit inflammation by very different biochemical mechanisms, they share the capacity to inhibit the first step in inflammation: leukocyte-endothelial adhesion. By better understanding the mechanisms by which these agents diminish inflammation, we hope to be able to design safer and more effective antiinflammatory agents for use in the treatment of the rheumatic diseases.

LEUKOCYTE-ENDOTHELIAL INTERACTIONS

The first steps in the pathogenesis of inflammation are adhesion of leukocytes to and transmigration across the vascular endothelium. Both leukocytes and endothelial cells play an active role in this process by regulating the expression and conformational alteration of the molecules that mediate adhesion. Many of the molecules that mediate this process have recently been identified, and their fine structure has been elucidated. Three major families of proteins that play a role in leukocyte-endothelial interactions are expressed on the surface of either leukocytes or the endothelium. The integrins are a family of heterodimeric adhesive proteins expressed on leukocytes. Monocytes and lymphocytes express the β_1-integrin VLA-4 (CD49d/CD29). All leukocytes express one or more of the β_2-integrins, a group of related heterodimeric adhesive proteins that share a common β chain (CD18) but differ with respect to their α chains (CD11a,b,c). Integrins bind to proteins on the surface of the endothelium (and other cells) that belong to the immunoglobulin superfamily (ICAM-1, ICAM-2, and VCAM-1). The selectins are the third family of adhesive proteins; they mediate adhesion by binding to carbohydrate residues on glycoproteins and glycolipids. Three distinct molecules comprise the selectin family; P-selectin (GMP-140 or PADGEM), E-selectin (ELAM-1), and L-selectin (LAM-1 or LECAM). P-selectin is expressed on stimulated platelets and endothelium, L-selectin on leukocytes (neutrophils, monocytes, and a subset of lymphocytes), and E-selectin on stimulated endothelium. The genes encoding these molecules are all located on chromosome 1 and appear to have arisen via duplication from a single gene. The selectins all possess an extracellular C-type (Ca^{2+}-dependent) lectin domain (responsible for binding to their cognate ligands), an epidermal growth factor (EGF)–receptor domain, and variable numbers of short consensus repeats (complement binding domains) in their extracellular portions, a hydrophobic transmembrane domain and a short cytoplasmic tail. E-selectin and P-selectin both bind to glycoproteins and glycolipids that contain Sialyl Lewis X antigen (a complex carbohydrate). Sialyl Lewis X antigen is expressed predominantly on the surface of neutrophils (reviewed in 1–4).

NONSTEROIDAL ANTIINFLAMMATORY DRUGS

Aspirin and aspirin-like drugs are the most commonly used antiinflammatory drugs. In one chemical form or another, these drugs, which are commonly grouped as nonsteroidal antiinflammatory drugs (NSAIDs), have been used for hundreds of years to treat a wide variety of inflammatory diseases. There was no convincing explanation for the clinical efficacy of aspirin, which is among the oldest NSAIDs still in use, until 1971 when Vane first proposed the hypothesis that the antiinflammatory effects of aspirin were due to its capacity to inhibit prostaglandin synthesis (5). By the end of the 1970s, Vane and his associates had amassed convincing evidence that the prostaglandin hypothesis of aspirin action was largely correct. They pointed out that almost all aspirin-like drugs (by then generally called nonsteroidal antiinflammatory drugs, or NSAIDs) inhibited prostaglandin synthetase and that the potency of these drugs (ID_{50}) in this regard paralleled their clinical potency or their effect in experimental animals, e.g. aspirin was anywhere from one fortieth to one two-hundredth as active as indomethacin and from one fifth to one fiftieth as active as ibuprofen. That over 40 NSAIDs have reached the clinic and that each of them at one dose or another inhibits prostaglandin synthesis is perhaps the best evidence that Vane's hypothesis is correct. All of these agents were found and developed as a result of their capacity to inhibit prostaglandin synthesis.

Cyclooxygenases and Lipoxygenases in Inflammation in Vivo

Recent studies have documented that there are actually two prostaglandin H_2 synthase isozymes (COX-1 and COX-2) that share 62% homology at the message and protein level (reviewed in 6). Despite the close relationship of the cDNAs for COX-1 and COX-2, the mRNA for these two isozymes differ greatly with respect to size, 2.8–3.0 kb and 4.1 kb for COX-1 and 2, respectively. The major difference between these two isozymes is that COX-1 is present in many cells constitutively, but COX-2 is expressed only after it is induced by tumor necrosis factor–α (TNF-α), interleukin-1 (IL-1), or lipopolysaccharide (LPS). Recent X-ray studies have demonstrated that aspirin irreversibly acetylates Ser 530 of COX-1, thereby blocking access of arachidonic acid (20:4) to the active site. Other NSAIDs sterically hinder access of 20:4 to the active site. By unknown means, aspirin inhibition of COX-2 leads to the generation of 15-hydroxyeicosatetraeonoic acid (15-HETE), albeit in the R form. A potential metabolite, 14,15-diHETE, is a potent inhibitor of neutrophil O_2^- generation. Engagement of two lipoxygenases (5- and 15-lipoxygenase) leads to the synthesis of lipoxins A_4 and B_4, which inhibit several leukocyte functions (7). In contrast, linolenic acid is converted by endothelium to 13-hydroxyoctadecadienoic acid (13-HODE) via the 15-lipoxygenase path-

way. 13-HODE is a potent chemorepellent, and studies have shown that when endothelial cells are activated by IL-1 to up-regulate their vitronectin receptor, 13-HODE synthesis is decreased: The ratios of 15-HETE to 13-HODE determined the adherence of tumor cells to endothelium via that receptor (8). Many NSAIDs selectively inhibit COX-1 or COX-2 (e.g. piroxicam, indomethacin, and sulindac sulfide are 10- to 40-fold selective for COX-1; 6-methoxy-2-naphthyl acetic acid, the active metabolite of nabumetone, is 15-fold selective for COX- 2), whereas other agents inhibit COX-1 and COX-2 equally (flurbiprofen, *S*-ibuprofen, meclofenamic acid); aspirin and sodium salicylate are equipotent with respect to COX-1 and COX- 2 but differ in their effects on platelet and gastric mucosa (9).

NSAID Actions Independent of Prostaglandins

The hypothesis that the locally produced prostaglandins lead to inflammation has been only partly substantiated. Although Vane's proposal that all NSAIDs inhibit the transformation of arachidonic acid to stable prostaglandins (PGs) (i.e. PGE_2 and PGI_2) has turned out to be largely correct, we still cannot generalize this proposition to all products of the arachidonic acid cascade and to all NSAIDs at all dosages. The three major antipyretic, analgesic drugs exert diverse effects on prostaglandin biosynthesis. When used to treat rheumatic diseases in dosages of 4 to 8 g per day, aspirin has antipyretic, antiinflammatory, and analgesic effects, and can inhibit the synthesis of prostaglandins in disrupted cell preparations. At the intermediate dosage indicated for analgesia (650 mg every 3 to 4 h), aspirin has antipyretic and analgesic but not antiinflammatory activity. And at its lowest clinical dosage (80 to 325 mg per day), aspirin exerts only its antiplatelet effect. Plasma levels of salicylate in individuals given intermediate, analgesic doses of aspirin can inhibit prostaglandin biosynthesis in vivo by kidneys, platelets, and vascular endothelium, whereas the low levels of aspirin that prevent thrombosis only affect prostaglandin synthesis by platelets. In contrast, higher plasma concentrations (18 to 30 mg per deciliter) are required to achieve an antiinflammatory effect (reviewed in 10). Those observations suggest two possibilities: Either the PGH synthase of cells that provoke inflammation is relatively insensitive to aspirin, or aspirin has a mode of action in addition to inhibition of prostaglandin biosynthesis and to which it owes its antiinflammatory property.

Further evidence that aspirin-like drugs exert clinical effects that do not depend on inhibiting prostaglandin biosynthesis can be drawn from the properties of sodium salicylate and acetaminophen. Although sodium salicylate shares many of the properties of aspirin, it fails to inhibit prostaglandin biosynthesis in disrupted cell preparations at concentrations that may be achieved in plasma (approximately 5 mM). Moreover, clinical studies show that because nonacetylated salicylates do not inhibit platelet function in vitro or ex vivo,

they do not cause bleeding. Indeed, acetaminophen, which also fails to inhibit prostaglandin biosynthesis, does not affect platelet aggregation, nor is it by any means antiinflammatory. We must therefore conclude that pain and fever can effectively be reduced without inhibiting the synthesis of prostaglandins at all (reviewed in 10).

Pro- and Antiinflammatory Properties of Prostaglandins

The Vane hypothesis is further weakened by findings that stable prostaglandins (PGE_1, PGE_2, PGI_2) possess not only proinflammatory but also antiinflammatory properties. These compounds produce vasodilation, act in synergy with complement component C5a or leukotriene B_4 to produce edema, mediate fever and myalgia in response to interleukin-1, and act in synergy with bradykinin to provoke pain. They also inhibit the function of T suppressor cells. All of these are proinflammatory effects of prostaglandins (reviewed in 10).

On the other hand, high doses of these stable prostaglandins inhibit inflammation in animal models of arthritis, while much lower doses inhibit inflammation induced by local skin irritants. Since the early 1970s we have known that PGI_2 and stable prostaglandins of the E type inhibit the in vitro activation of neutrophils, platelets, and mononuclear phagocytes by interfering with their stimulus-response coupling (11). NSAIDs increase cellular cAMP in these cells, levels of which are regulated via prostaglandin receptors. The relevance in vivo of these data obtained in vitro is supported by the observation that experimental arthritis or glomerulonephritis in rats can be reduced with systemic PGE_1 (12–14). These are antiinflammatory effects of prostaglandins.

As a class, NSAIDs are planar, organic anions that partition across the lipid bilayers of plasma membranes in accordance with the Nernst equation. The more acidic the pH (as at inflammatory sites) the greater the proportion of the lipophilic form of NSAIDs, which subsequently interfere with cell function [including assembly of a superoxide anion-generating system by a cell-free, membrane-rich preparation from neutrophils (15)], the activity of phospholipase C in mononuclear cells, the 12-hydroperoxyeicosatetraneonic acid peroxidase in platelets, and signal transduction in neutrophils and lymphocytes (reviewed in 10).

Miyahara & Karler found that the first effect of aspirin-like drugs on cell metabolism was the uncoupling of oxidative phosphorylation by isolated mitochondria (16). More recent studies have shown that aspirin (but not acetaminophen) alters the uptake of precursor arachidonate and its insertion into the membranes of cultured human monocytes and macrophages. Salicylates also inhibit anion transport across a variety of cell membranes. Again, the capacity of salicylates to inhibit anion movements is not shared by acetaminophen. Finally, NSAIDs inhibit synthesis of cartilage proteoglycan and bone metabolism (both in vitro and in vivo) by mechanisms that do not depend on

inhibiting the PGH synthase. It is a matter of clinical concern that some classes of NSAIDs (e.g. salicylates), but not all (e.g. piroxicam), inhibit proteoglycan synthesis, thereby promoting loss of cartilage matrix.

NSAIDs Interfere with Neutrophil Functions

Recent work has shown that aspirin-like drugs affect stimulus-response coupling in the most abundant cells of acute inflammation: neutrophils. Neutrophils injure tissues by releasing proteases; inflammatory peptides; reactive oxygen species, such as O_2^- and H_2O_2; and lipid irritants, such as platelet-activating factor and leukotriene B_4. Activation of the neutrophil in response to soluble stimuli (chemoattractants) or to immune complexes follows general pathways of stimulus-response coupling secretory cells and is inhibited by all known NSAIDs.

NSAIDs inhibit the cell-cell aggregation of human neutrophils induced by chemoattractants and mediated by the cell surface adhesion molecule CD11b/CD18. Although all NSAIDs inhibit aggregation, only some inhibit enzyme release and/or O_2^- generation. Millimolar concentrations of sodium salicylate and of aspirin (levels achieved in treating rheumatoid arthritis or rheumatic fever) are required to inhibit the aggregation of neutrophils. However, at these concentrations, sodium salicylate does not interfere with the activation of platelets or synthesis of thromboxane A_2. In contrast, aspirin at one-tenth to one-hundredth these concentrations inhibits platelet aggregation and completely inhibits thromboxane biosynthesis via its effect on PGH synthase. Therefore, the shared antiinflammatory effects of aspirin and sodium salicylate are likely related to their common inhibition of neutrophil activation rather than to their divergent actions on prostaglandin biosynthesis. In contrast to aspirin and sodium salicylate, acetaminophen does not affect neutrophil aggregation.

Inhibitory effects of NSAIDs on neutrophil activation in vitro can also be demonstrated in the clinic. Indeed, neutrophils derived from the synovial fluid of patients with rheumatoid arthritis produced less O_2^- following 10 days of therapy with piroxicam than cells from normal volunteers who were given ibuprofen or piroxicam for 3 days; these cells failed to aggregate normally in response to chemoattractants. Sodium salicylate, an ineffective inhibitor of PGH synthase in vitro, is as effective as aspirin at inhibiting neutrophil activation.

A Novel Biochemical Mechanism for NSAIDs: NSAIDs Uncouple Chemoattractant Receptors from Their Cellular Transduction Mechanisms

At antiinflammatory concentrations, NSAIDs appear to uncouple receptors from their effector molecules in the plasmalemma, including those regulated

by at least one guanine nucleotide–binding (G) protein. Pertussis toxin, via its capacity to ADP-ribosylate the alpha subunit of some plasma membrane G proteins (and thereby abrogate their function), interferes with signal transduction in a variety of cells, including the neutrophil. Compared to pertussis toxin, sodium salicylate alone only modestly inhibits the production of O_2^- induced by chemoattractants; it inhibits aggregation to a far greater extent. However, sodium salicylate blocks the inhibitory effect of pertussis toxin on neutrophils: Cells coincubated with both pertussis toxin and sodium salicylate could still generate superoxide anion by inhibiting pertussis toxin. This paradoxical effect of salicylate suggests that salicylates interfere with the action of pertussis toxin near the site of its interaction with the α subunit of the G protein. NSAIDs (salicylate, piroxicam, and indomethacin) block the pertussis toxin–dependent ADP-ribosylation of the G protein in purified neutrophil membranes, and salicylates and piroxicam inhibit, in part, the pertussis toxin–sensitive formation of diacylglycerol that follows cell activation (10, 15, 17, 18).

CORTICOSTEROIDS

Since their first use in clinical medicine, corticosteroids have remained among the most widely used drugs for the treatment of inflammatory diseases. The broad range of inflammatory illnesses for which corticosteroids are effective therapies has suggested that corticosteroids might have multiple mechanisms of action, a hypothesis borne out by the large number of empirically defined antiinflammatory effects of glucocorticoids. Although glucocorticoids are among the most potent and widely used antiinflammatory agents, the mechanisms by which they reduce inflammation are not completely understood. Various hypotheses have been proposed; these include allosteric effects on proteins (19), redirection of lymphocyte traffic (19–26), direct inhibition of various phospholipases (27), the induction of such proteins as lipocortin (27–31), inhibition of the transcription of various cytokines (IL-1, IL-3, and TNF-α) by endothelial and other inflammatory cells (33–40), metalloproteases and inflammatory enzymes (inducible nitric oxide synthase) (41-44), and our own earlier suggestion that glucocorticoids stabilize lysosomal and other cellular membranes (45, 46). None of these hypotheses are sufficient to account for such well-known pharmacological effects of glucocorticoids in humans: leukocytosis (47); inhibition of leukocyte recruitment to inflamed areas (48, 49); retention of lymphocytes in the lymphatic circulation, with shrinkage of peripheral lymph nodes (25, 26); and the promotion of microbial infection (21, 26).

An alternative explanation for the antiinflammatory effects of glucocorticoids is suggested by recent experiments in our laboratory in which we found that glucocorticoids diminish the capacity of the endothelium to express

adhesive molecules for leukocytes. Perhaps the most important advance in our understanding of the inflammatory process has been the demonstration that, in response to inflammatory cytokines and endotoxin, the vascular endothelium plays an active role in the recruitment of leukocytes to inflamed and infected sites. During the past decade the molecular basis for the interaction between endothelium and leukocytes has been explored in detail and many of the molecules responsible for the adhesion of leukocytes to endothelium at inflamed sites have been identified (reviewed in 4). We demonstrated that glucocorticoids act at the transcriptional level to diminish cytokine- and endo- toxin-stimulated endothelial expression of the adhesion molecules E-selectin and ICAM-1 (50). This observation also may explain the clinical observation that steroid therapy is associated with leukocytosis (lack of leukocyte-endothelial adhesion in the microvasculature), redistribution of lymphocytes (diminished lymphocyte-endothelial interactions), and enhanced susceptibility to infection (inability to recruit leukocytes to infected sites). Thus our findings suggest the general hypothesis that corticosteroids act as antiinflammatory agents by diminishing, both directly (decreased expression of adhesive molecules) and indirectly (diminished cytokine production), the ability of endothelial cells to direct leukocyte traffic into inflamed or infected tissue.

METHOTREXATE

Methotrexate is an antifolate that is commonly administered in low doses at weekly intervals to treat such inflammatory diseases as rheumatoid arthritis. Although originally developed as an anticancer agent because of its capacity to block purine and pyrimidine synthesis in malignant (and normal) cells, methotrexate at very low dosage is now one of the most widely used second-line agents in the therapy of rheumatoid arthritis. Despite its known antifolate properties, there is no evidence that cytotoxicity caused by blockade of purine and pyrimidine synthesis in inflammatory cells (or lymphocytes) is responsible for the antiinflammatory effects of methotrexate. Indeed, repletion of folate with either high-dose folate supplementation or reduced folate (citrovorin) does not interfere with the antirheumatic effects of methotrexate, although folate repletion does protect against methotrexate-mediated toxicity. Other antiinflammatory effects of methotrexate include inhibition of delayed-type hypersensitivity, inhibition of neutrophil accumulation, inhibition of neutrophil (but not mononuclear cell) leukotriene B_4 production, antagonism of IL-1 activity, and inhibition of angiogenesis. With the exception of the effect of methotrexate on angiogenesis, the antiinflammatory effects of methotrexate can only be demonstrated in in vivo models of inflammation.

The Antiinflammatory Activity of Methotrexate: A Biochemical Hypothesis

Our laboratory has recently examined the biochemical mechanism for the antiinflammatory effects of methotrexate and found evidence to suggest a novel mechanism for the antiinflammatory effects of methotrexate; adenosine mediates these antiinflammatory effects. Methotrexate is taken up by cells in which it is rapidly polyglutamated. The polyglutamated derivatives accumulate in cells and tissues where they may still actively inhibit folate-dependent enzymes (51–53). Among other effects, methotrexate promotes the accumulation of dihydrofolate polyglutamates (52, 54). Both dihydrofolate polyglutamates and methotrexate polyglutamate inhibit the enzyme AICAR (5-aminoimidazole-4-carboxamide ribonucleotide) transformylase, potentially leading to intracellular accumulation of AICAR (52, 54). The intracellular accumulation of AICAR enhances adenosine release from stressed cells (55, 56), which leads to the interesting hypothesis that adenosine, a well-known endogenous inhibitor of inflammation, may actually be the mediator of the antiinflammatory effects of methotrexate. Cronstein et al (57) have tested this hypothesis in an in vitro system and observed that treatment of endothelial cells or fibroblasts with methotrexate led to a modest increase in adenosine release. However, a much greater increase in adenosine release was observed when the methotrexate-treated cells were exposed to a stress (in this case activated neutrophils). Fewer stimulated neutrophils adhered to methotrexate-treated endothelial cells and fibroblasts in this in vitro model of inflammation, and the diminished neutrophil adhesion was due to the increase in adenosine release from the methotrexate-treated cells. A similar increase in adenosine release and an adenosine-mediated decrease in inflammatory cell-cell interactions were observed when fibroblasts and endothelial cells were treated with AICARiboside, a cell-soluble precursor of AICAR. Recent studies by Asako and coworkers (58) confirmed this hypothesis in vivo; methotrexate applied topically (and in relatively high concentration) diminished leukocyte extravasation from the vasculature via a mechanism that is dependent upon the presence of adenosine; adenosine deaminase reversed the antiinflammatory effect of methotrexate. Moreover, using specific antagonists, these investigators demonstrated that the antiinflammatory effects of methotrexate, acting via adenosine, were due to interaction with adenosine A_2 receptors (presumably on the leukocytes) (58).

More recently Cronstein et al (59) have demonstrated that weekly treatment of mice with pharmacologically relevant doses of methotrexate increases splenocyte AICAR content, increases adenosine concentration in inflammatory exudates, and inhibits leukocyte accumulation in inflammatory exudates (murine air-pouch model of inflammation) by an adenosine A_2 receptor–mediated

effect. Similarly, Gruber et al have shown that intravenous infusions of AICARiboside also diminished leukocyte accumulation and cardiac injury in a model of ischemic injury (reperfusion injury), although the role of adenosine in the inhibition of cardiac injury is less clear in this model (60). Because sulfasalazine, an antiinflammatory agent developed to treat rheumatoid arthritis and more commonly used to treat inflammatory bowel disease, may also interfere with AICAR metabolism (61), Baggott and coworkers (62) have suggested that sulfasalazine may diminish inflammation via adenosine release in a manner similar to methotrexate.

The Antiinflammatory Effects of Adenosine in Vitro

Cronstein et al (63) and Newby et al (64) first demonstrated that suspensions of neutrophils release adenosine into the extracellular milieu. Removal of this endogenously released adenosine led to a significant increase in neutrophil responses to chemoattractants (63). Subsequent studies have confirmed these early observations (65). Other cells may release adenosine into extracellular milieu, such as vascular endothelium. In 1986 we showed that both an exogenously applied adenosine receptor agonist (2-chloroadenosine) and endogenously released adenosine protected human vascular endothelial cells from injury by stimulated human neutrophils (66). These observations have subsequently been confirmed by Gunther & Herring (67) and have suggested that adenosine might be a critical regulator of inflammation. Moreover, these results suggested that increased adenosine release could be utilized to diminish inflammation. Interestingly, the existence of a potential counter-regulatory effect of extracellular adenosine deaminase (released from dead cells or bacteria) was inferred from the observation that adenosine deaminase can bind to opsonized particles (zymosan) and, by metabolizing extracellular adenosine to inosine, may promote phagocytosis and oxygen radical generation by activated leukocytes (68). Perhaps as important as the effects of adenosine on the cellular mediators of inflammation is its capacity to inhibit the stimulated secretion of TNF-α (69, 70).

The Antiinflammatory Effects of Adenosine in Vivo

Adenosine and its analogues are potent inhibitors of inflammation in two different animal models (61, 71, 72). Green and colleagues have reported that a single daily dose (intraperitoneal) of adenosine reduced the severity of experimental adjuvant arthritis in rats (61), a finding that is quite surprising considering the extremely rapid metabolism of adenosine ($T_{1/2}$ in whole blood $\geq$ 1 min; BN Cronstein, unpublished data). Although adenosine diminishes leukocyte function via A_2 receptors and, in general, promotes inflammatory functions via A_1 receptors, Schrier and coworkers (71, 73) have observed that adenosine A_1 receptor agonists are better inhibitors of pleural and peritoneal

inflammation than A_2 agonists, when studied in vivo. It is unclear whether the apparent A_1 specificity is a result of individual characteristics of the agonists used, which, although possessing higher affinity for A_1 than A_2 receptors, are not absolutely specific. Indeed, subsequent studies using adenosine receptor antagonists that are highly specific for their subtype suggested that adenosine modulates inflammation in vivo via A_2 receptors (58).

CONCLUSIONS

We have reviewed the mechanisms of action of three different classes of antiinflammatory agents: aspirin-like nonsteroidal antiinflammatory agents, steroids, and methotrexate. Although in clinical use these agents are often used together and appear to be complementary, these three different types of agents share two major characteristics. All three classes of antiinflammatory agents diminish, by widely varying mechanisms, (*a*) the endothelial-leukocyte and/or leukocyte-leukocyte interactions required for inflammation and (*b*) the production of multiple soluble mediators of inflammation, which mediate and amplify the inflammatory process. It is clear from the remarkable redundancy of inflammatory mediators and adhesive molecules responsible for recruitment of leukocytes to inflamed sites that in order to successfully diminish inflammation, an agent must inhibit the inflammatory process at more than one step. Although interference with one type of inflammatory mediator or adhesive molecule may be an effective approach to the development of new antiinflammatory agents, it is unlikely that "mono-specific" antiinflammatory agents will have more than limited success in the therapy of rheumatoid arthritis. The multi-pronged attack on inflammation by the three classes of antiinflammatory agents reviewed may serve as a model for the development of new classes of antiinflammatory agents.

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