NONSTEROID ANTI-INFLAMMATORY AGENTS 6556

H. E. PAULUS AND M. W. WHITEHOUSE

Department of Medicine, University of California School of Medicine, Los Angeles, California

INTRODUCTION

We must begin this review by reiterating the words of the previous reviewers of this subject in these Reviews, published three years ago: "There has been no truly dramatic breakthrough in our understanding of inflammation or anti-inflammatory drugs in recent years" (1). A similar conclusion was expressed two years later by the (last) reviewers of this subject for the Annual Reports in Medicinal Chemistry, "Our overall impression is that there have been no really significant advances in the past year. An encouraging sign, however, is the increasing amount of research that is being directed toward better understanding of the inflammatory processes, in hopes that from this work will come the breakthrough so badly needed in this field" (2).

One development, at least, is the blurring of the distinction between anti-inflammatory agents and immunosuppressant drugs, with (*i*) the clearer realization that certain chronic inflammatory states in man and experimental animals may be initiated or sustained by malregulated immunocompetent cells and (*ii*) the increasing recognition that some of the immunoregulatory drugs may confer great clinical benefit in both human disease and experimental animal models characterized by progressive debilitation with chronic inflammation (arthritis, demyelinating disease, myositis, etc.).

Animal models of acute induced inflammation were discussed in one of these *Reviews* by Winter in 1966 (3). During the past decade (anti-) edema assays have been used to screen thousands of chemicals. Among those that showed anti-edemic activity, several dozen are now in various stages of clinical evaluation. Their activity in patients is generally similar to that of aspirin and phenylbutazone (which were initially used to validate the animal assay methods.) Demonstration of the anti-rheumatic properties of these new drugs in patients during the past few years establishes to some degree the validity of the rat edema assays for selecting moderately effective nonsteroidal "anti-inflammatory" drugs (NSAIDs).

However, as more new NSAIDs reach general use, the clinical need for additional similar compounds will decrease, and it will then be imperative to develop truly new classes of agents that are either more effective or less toxic than presently available drugs. For this purpose, new biological assays are almost certainly needed.

A plausible formulation of the pathogenic events occurring in the inflamed joints of patients with rheumatoid arthritis has been pieced together by a number of workers and eloquently presented by Zvaifler (4) and Ziff (5). These events involve the combination within the joint of an antigen (gamma globulin) with an antibody (rheumatoid factor) and complement, causing the release of chemotactic factors, which attract leukocytes to the area. The leukocytes phagocytize the antigen-antibody-complement complexes and also release into the joint the many enzymes contained in their lysosomes. These lysosomal enzymes then cause cartilage and tissue injury and magnify the inflammation.

Alternative viewpoints consider rheumatoid arthritis to be a disorder of cell-mediated immunity (6), and inflammation to be subject to neural and endocrine factors (7).

Thoughtful reviews, with valuable bibliographies, by two of the fathers of anti-inflammatory pharmacology (8, 9) deserve intensive study. The early history (10), cellular pharmacology (11), and chemistry (12, 13) of NSAIDs; the effect of salicylates on various enzymes (14) and drug design for chronic inflammatory disease (15) have been discussed in depth. A forthcoming multi-authored book surveys the chemistry and pharmacology (both clinical and animal) of NSAID and corticosteroids (16).

Space restriction has unfortunately precluded considerations of drug action on leukocytes (other than platelets), connective tissue, pain, disease, or various mediators and enzyme systems associated with inflammation or tissue injury, and clinical studies with NSAIDs; notwithstanding the fact that each of these topics has engendered a considerable literature over the past three years.

PROSTAGLANDINS

Prostaglandins (PGs) are pharmacologically potent lipids widely distributed in mammalian tissues and body fluids (17), first described and named by Von Euler in 1936 (18). During the past decade adequate amounts of prostaglandins for extensive biological evaluation were produced from essential fatty acids, utilizing PG-synthetases from seminal vesicles (19). They act locally in the tissues where they are produced, and since they are rapidly inactivated, may be considered "local hormones" (20). Prostaglandins have been the subject of recent Annual Reviews (21, 22); Pharris & Ramwell have edited a book devoted to the role of prostaglandins in cellular biology and inflammation (23).

Recently, the possible role of E-type prostaglandins (PGEs) in inflammation has been elucidated, and NSAID have been found to inhibit the production of PGE_2 from arachidonic acid. Intradermal injections of PGE_1 and PGE_2 in nanogram doses produced a local increase in vascular permeability in guinea pig (24) and rat skin (25, 26) and induced a weal and flare response in the human forearm (25). Their inflammagenic potency is comparable with that of histamine, bradykinin, and 5-hydroxytryptamine. PGE-type activity has been identified in the inflammatory exudate of carrageen-induced inflammation in the rat (27, 28), occurs relatively late in the sequence of inflammatory mediators, and is temporal-

ly associated with the migration of leukocytes into the inflamed area (29). Indeed, PGE_1 has been reported to have leukotactic properties when rabbit leukocytes were studied in Boyden chambers (26, 30). Prostaglandin-like activity has also been demonstrated in inflamed human skin (31–33). Prostaglandins were released from rabbit leukocytes during phagocytosis (34) and their production may be related to the release of phospholipases from leukocyte lysosomes during endocytosis (35).

Aspirin, indomethacin, and salicylate have been shown by a number of workers to prevent the synthesis or release of PGE_2 and $PGE_{2\alpha}$ from arachidonic acid in a variety of tissues, including extracts of guinea pig lung (36, 37), human platelets (38), dog spleen (39), extracts of sheep (40) and bovine (41) seminal vesicles, human semen (42), and mouse ascites tumor cells (43). Inhibition of PG-synthetase(s) can be demonstrated at drug concentrations readily attained in the serum of patients taking the usual therapeutic doses, and has also been demonstrated with mefenamic acid, phenylbutazone, clofibrate, and naproxen (41, 44). The relative potency of NSAIDs as inhibitors of prostaglandin synthetase approximates their order of potency against carrageenin-induced inflammation, and is especially marked for certain D and L isomers (41), which are not otherwise readily distinguished in other in vitro biochemical assays (albumin binding, uncoupling of oxidative phosphorylation, etc.). However an exception to the correlation of PG-synthetase inhibition with anti-inflammatory activity has been noted with certain arylacetic acids (43). Aspirin did not affect the inflammation produced when PGE_1 was injected intradermally in humans, indicating that the effect of NSAIDs is on PGE synthesis or release, rather than a direct antagonism of formed PGE (45). By contrast, the corticosteroids and immunosuppressive drugs are relatively inactive, although a decrease in PGE_2 and $PGF_{2\alpha}$ synthesis has been reported when hydrocortisone was added to rat skin homogenates (46). It has even been suggested that the common gastrointestinal toxicity of NSAIDs may be explained by their suppression of PGE synthesis, since PGE_1 has been reported to inhibit gastric secretion (47, 48). Fears have been expressed that aspirin may negate the contraceptive function of intrauterine devices (believed to stimulate PG production in utero). To date, there is no solid evidence to justify these fears, but clarification is urgently required lest the therapeutic value of aspirin be prejudiced needlessly.

The evidence supporting the hypothesis that the anti-inflammatory effects of NSAIDs may be due to their suppressive effect on prostaglandin synthesis has been eloquently reviewed and formulated by Vane (36, 44, 49) and by Willis and his associates (50). It should be noted, however, that high doses of PGE_1 and PGE_2 may suppress the inflammation of adjuvant arthritis as well as that induced by carrageenin in the rat (51–53); although the doses used cause adrenal hyperplasia, prostration, and diarrhea, which may be responsible for the observed anti-inflammatory effects. In any event, the interactions of the E type of prostaglandins, inflammation, and anti-inflammatory drugs seem unlikely to direct us toward a new class of anti-inflammatory drugs, but may certainly increase our understanding of the NSAIDs presently available.

LYSOSOMES

Drug action on lysosomes has been intensively studied over the last few years. This plethora of reports can be strongly criticized on several counts.

A. It is worth recalling that the original concept of the lysosome arose from observations of structure-linked latency of acid hydrolases, this latency being due to a membrane-like barrier limiting the accessibility of an enzyme to its substrate. De Duve has taken pains to emphasize that "the lysosome is not really a *body* as its name suggests; it is part of a *system*, and can be viewed in the right perspective only if the system itself is understood" (54). Much of the confusion in the literature must be attributed to misunderstanding the role of the lysosome within a physiological self-regulating system.

B. Various etiologies of human arthritis (55–58) and rat adjuvant disease (59) have stressed the potential destructive capacity of acid hydrolases liberated from within the endogenous cellular elements of the connective tissue or derived from infiltrating leukocytes, a viewpoint that has inevitably biased the study (or at least the interpretation) of drug-lysosome interactions. The serum or inflamed paws of adjuvant arthritic rats undoubtedly contain higher levels of catabolic enzymes than are to be found in normal animals (59-62); some of these enzymes are elsewhere found associated with lysosomes, e.g. acid hydrolases such as β-glucuronidase, acid phosphatase, or lysozyme. The time at which these serum enzymes are maximal (61) does not always coincide with the onset of tissue damage. The assumption that the injury is a consequence of the presence in serum of potentially destructive enzymes is certainly not warranted on this evidence alone. Moreover, catabolic activity is generally not the principal feature of rheumatoid arthritis and what damage the articular cartilage suffers is probably mainly a consequence of the proliferative activity of synovial tissue cells (including adopted leukocytes).

C. Experimental studies of the action of drugs and hormones on enriched lysosome fractions (reviewed 63–66) have particularly centered on the anti-inflammatory corticosteroids and chloroquine and more recently the catecholamines and ATP (67, 68), which retard or diminish the release of acid hydrolases from lysosomal preparations submitted to a form of "shock" in vitro (irradiation, detergent, increase in temperature, reduction in tonicity, pH, etc.). Some of the data reported (e.g. 14% inhibition of enzyme release from shocked rabbit liver lysosomes with 0.5 mM steroid) make it difficult to believe the author's claim that "Synthetic anti-inflammatory steroids share the common property with the naturally occurring hormones of stabilizing lysosomes" (69). Other types of drugs that stabilize lysosomes under these conditions in vitro, such as stilbamidine (70) or phenothiazines (65) have so far not been found to display cortisol- or chloroquine-like antiarthritic activity. Other reports have failed to confirm that steroids and chloroquine stabilize lysosomes (71). Attention has been drawn to the biphasic effects of steroids, alcohols, and phenothiazine on various biological membranes (72), including rat-liver lysosomes (73), which appear to be associated with surface-active and membrane-expanding properties of these compounds; thus it

becomes difficult to classify drugs strictly as stabilizers or labilizers, with factors such as concentration and solubility rather than structural chemical specificity being of cardinal importance.

D. Liver lysosomes from adjuvant-arthritic rats released acid phosphatase and β-glucuronidase more slowly than normal rat lysosomes (74); since two anti-arthritic agents, aspirin and cycloleucine (but not phenylbutazone), given in vivo actually increased enzyme release from adjuvant-diseased liver lysosomes, it was suggested that membrane labilization "may quench the expression of chronic inflammation by eliminating antigenic debris and materials which evoke hyperplasia". Labilization of enzyme release from lysosomes has been reported when NSAIDs are added in vitro to preparations of lysosomes from rat and rabbit liver (65, 75–77), rat or rabbit blood leukocytes, and rabbit peritoneal PMN leukocytes (78). These paradoxical (i.e. unsteroid-like) effects of the NSAID are all the more remarkable considering that many of these experiments have dealt with liver lysosomes heavily contaminated with mitochondria, which have a high binding capacity for these acidic drugs and must very effectively compete with the lysosomes in taking up drug from solution. After administration in vivo at effective anti-inflammatory dosages, it is reported that phenylbutazone and indomethacin *failed* to stabilize rat liver lysosomes (79), phenylbutazone and aspirin *did* stabilize these lysosomes (80), and chloroquine labilized liver lysosomes (81).

The embryotoxic activity of sodium salicylate has been ascribed to lysosomal labilization, promoting glycosaminoglycan degradation (82). Yet other reports make it abundantly clear that despite earlier claims to the contrary (83), salicylates do not stabilize liver lysosomes (64, 84–86) and may even counteract any stabilizing effect of cortisol (87). Another interpretation of possible lysosome labilization by AID, including high levels of steroids (73), is that it may provide a basis for the ulcerogenic action of both steroids and the NSAID.

E. Inhibition of lysosomal enzymes once released, has been demonstrated with relatively high levels of NSAID (88, 89) and sodium aurothiomalate (77, 90). This gold preparation appears to block thiol groups on the acid hydrolases with K_i's approximately 10^2 that of orthodox thiol reagents (mercurials, iodoacetate). Acid hydrolases from rat liver (91) or human synovial fluid leukocytes (90) were similarly depressed after adminstration of aurothiomalate in vivo.

F. α-Globulin-like macromolecules present in pregnancy serum (92) or the serum of adjuvant-arthritic rats (93) are reported to stabilize isolated liver lysosomes. Whether these fractions are part of the natural inflammalytic system is presently unclear. It is worth reiterating the viewpoint (55) "Perhaps it is time to give more attention to effects of stabilization, or enzyme deficiency or inhibition, as possible factors in chronic inflammatory states."

G. The bizarre difference in the drug-response of peritoneal PMN leukocytic granules from rabbits (78) and guinea pig (94), the former being labilized and the latter stabilized by NSAID (excluding corticosteroids), emphasizes the critical need to have much more data concerning in vitro and in vivo drug actions on lysosomes of human origin, for constructing less fragile theories concerning the mechanism of action of NSAID. The "consistent" effect of chloroquine in

stabilizing PMN granules from both the rabbit and guinea pig indicates, however, that lysosome studies might be useful in screening for drugs, which, like chloroquine, are not readily detected in conventional anti-inflammatory assays. One theory of chloroquine action ascribes its function to raising the internal pH of the lysosome (95) rather than stabilizing its membrane.

PLATELETS

No attempt is made here to review the extensive recent literature on the effects of aspirin on platelets. Aspirin inhibits adenosine diphosphate (ADP) release from human platelets and the resultant aggregation induced by collagen. The second wave of platelet aggregation induced by ADP or epinephrine is also inhibited although the primary wave is not affected. The release of platelet-bound serotonin is also suppressed. As little as 300 mg of aspirin is effective, and its anti-aggregation effect is still detectable 4–6 days after aspirin ingestion. The similar effects of acetic anhydride and the substantially greater potency of aspirin than sodium salicylate, suggest that platelet acetylation is responsible for this effect of aspirin (96–100).

Other NSAIDs also affect platelets (96, 101, 102). Indomethacin is more potent than aspirin as an inhibitor of the second phase of epinephrine-induced aggregation, or ^{14}C-serotonin release induced by connective tissue, but the effect persists for only a few hours. Mefenamic acid, flufenamic acid, and phenylbutazone are effective at slightly higher concentrations than used with indomethacin, but much higher concentrations of salicylic acid are required for the same effect. The use of other NSAIDs after prior aspirin ingestion did not inhibit platelet aggregation to a greater degree than aspirin alone. This suggests that both NSAIDs and aspirin combine with a common receptor, but only aspirin persistently blocks this receptor.

Although the role of platelet aggregation in chronic inflammation is unclear at present, the remarkable similarity of the effects of various NSAIDs on platelets indicates that anti-aggregation activity should be evaluated when searching for new NSAIDs.

DRUG INTERACTIONS

As indicated above, one NSAID may interact with other NSAIDs in a way that does not enhance the pharmacological (anti-aggregation or anti-inflammatory) activity of the combination. Simultaneous administration of aspirin with indomethacin or phenylbutazone did not produce an additive effect on the carrageenin-induced rat paw edema (103). In fact, combined administration of any two of these drugs failed to produce any anti-inflammatory effect greater than that produced by either of them given alone at the same dose (104). However, the combination of hydrocortisone with one NSAID caused greater inhibition of paw edema than that caused by either drug alone (104). Low doses of aspirin actually decreased the anti-inflammatory effects of other NSAIDs (flufenamic acid, indomethacin, or phenylbutazone) on the secondary paw swelling of adjuvant disease (105, 106). Antagonism was also shown if aspirin administration preceded

administration of the other NSAID, suggesting that the antagonism was long-lasting. This antagonism among NSAIDs has been confirmed and extended to ibuprofen and acetaminophen, using somewhat different dosage schedules and endpoints for assessment (E. M. Glenn, unpublished). Chlorpromazine also antagonizes the effects of NSAIDs (105). The mechanism of antagonism is not clear. Salicylic acid decreased the serum level of ^{14}C-indomethacin in rats; concomitantly, fecal and biliary excretion of ^{14}C increased, urinary ^{14}C excretion decreased, and ^{14}C concentrations in tissues were modified (107). These effects occurred after either oral or intravenous administration of salicylic acid. Other agents such as phenylbutazone, chlorogenic acid, and acetic acid had no effect on plasma ^{14}C levels.

Lower serum ^{14}C levels were also found in 8 subjects with rheumatoid arthritis when ^{14}C-indomethacin was given orally with aspirin, compared to levels attained when the same dose of indomethacin was given without aspirin (108) However, another study with 8 normal subjects and 33 patients with arthritis failed to show any effect on serum levels of indomethacin when aspirin was added to chronic indomethacin therapy or when indomethacin was added to a baseline of aspirin therapy (109). In this latter study serum indomethacin concentrations were determined by a fluorometric method, perhaps explaining the difference from the results obtained with the ^{14}C-indomethacin.

No one has yet demonstrated in patients that the simultaneous administration of two NSAIDs decreases the anti-inflammatory effect of either of the drugs given alone. However, the Cooperating Clinics Committee of the American Rheumatism Association found that indomethacin was no more effective than placebo in rheumatoid arthritis, when it was added to a background of aspirin therapy (110).

There is therefore, no evidence to support the common clinical practice of adding a second NSAID, if the patient responds poorly to the first NSAID, and the simultaneous administration of two or more NSAIDs may actually be less efficacious than one alone.

Interactions of NSAIDs with other drugs have been repeatedly demonstrated. Thus probenecid decreases the renal excretion of ^{14}C-indomethacin, presumably due to competition for renal tubular secretion (111). Phenylbutazone, oxyphenbutazone, and salicylates displace a variety of other drugs from common protein-binding sites and may increase the activity (or toxicity) of sulfonamides, oral anticoagulants, tolbutamide and methotrexate (112–114). Phenylbutazone may induce its own metabolism by liver microsomal enzymes and that of several other drugs as well (112). Spironolactone (Aldactone) decreases the gastrointestinal toxicity of indomethacin in animals by enhancing its hepatic microsomal metabolism, substantially lowering plasma indomethacin levels, and thus decreasing the toxicity (115–118).

Albumin Binding

This topic continues to receive considerable attention, both in relation to the evaluation of compounds in vitro and as an experimental basis for exploring theories of the mechanism of action of the acidic NSAID. Newer methodologies

to determine the binding of NSAID and other acidic drugs to human serum albumin (HSA) include displacement of the fluorogens, dansylamide (119, 120), or N-dansylglycine (121), and studies of the drug-albumin complex using circular dichroism (121–123).

A rapid method for estimating drug-albumin affinity constants has been described. It enlists a Fortran program for computation and assumes one molecule of drug binds per molecule HSA; this assumption seems valid for phenylbutazone (124). Independent studies indicate that HSA has one strong binding site and two weaker ones for phenylbutazone (121) but three strong binding sites for flufenamic acid (122).

Acetylation of a lysyl residue in HSA, after ingestion of aspirin in vivo or by incubating HSA with aspirin in vitro, increases the binding of 3-acetamido-2,4,6-triiodo benzoate (125) and phenylbutazone, has no effect on dicumarol binding, but decreases that of flufenamic acid (123). These studies suggest that the plasma binding (and hence the pharmacokinetics) of a second NSAID such as phenylbutazone might possibly be altered by prior ingestion of aspirin. Competition by endogenous fatty acids with the albumin-binding of salicylates and phenylbutazone does not seriously interfere with drug binding except at levels of 2 mM or more free fatty acid in plasma (126).

The specificity of the NSAID in protecting bovine albumin (BSA) from heat denaturation has been detailed (127). NSAIDs were unable to preserve BSA from chemical denaturation (127) but did protect canine erythrocytes to some degree from detergent-induced, as well as thermal denaturation (128).

Chi-square analysis of the effect of 370 compounds on three properties of albumin in vitro (thiol-disulfide interchange, heat denaturation, aldimine formation with trinitrobenzaldehyde) and various in vivo systems for screening AID indicated a strong association between drug-albumin interactions and drug inhibition of bradykinin-induced bronchostriction and (pain response to) acute knee joint synovitis (129). Salicylate and phenylbutazone, but not indomethacin, are reported to bind to erythrocytes (130). Predictions of drug binding in the circulation should be preferably made on the basis of in vitro studies of drug binding to whole blood, erythrocytes, and plasma.

Other *caveats* have been uttered with respect to differences in salicylate binding to crystalline and fraction V preparations of bovine albumin (131) and dansylamide binding to various different commercial preparations of HSA (120). Species-differences in the binding of salicylate (132, 133), phenylbutazone, and indomethacin (134) to various serum albumins have been noted and ought to be investigated further with other NSAIDs, especially before attempts are made to correlate differences in drug activity in the different laboratory animals on the basis of rates of drug metabolism alone. Salicylate binding is particularly low in rat and dog (133), the two species often utilized for long term toxicity studies.

A comparison of the amino acid composition of HSA preparations from 30 individual patients with rheumatoid arthritis and 24 normal subjects indicated that the arthritic patients had an albumin containing 14% more phenylalanine (p 0.02) and 14% less lysine (p 0.01) than normal albumin (135). If this truly

represents a change in polypeptide composition (and is not due to binding of extraneous amino acids) the binding of anionic NSAIDs might be affected, as one prime site of binding may be the cationic ε-amino groups of lysine (136).

A provocative theory attributes the efficacy of NSAIDs in human arthritis to their ability to displace ill-defined peptides from HSA, these peptides being supposed to be anti-inflammatory (137). This argument is advanced on the basis of (*i*) the fact that arthritic patients have abnormally high binding of these peptides to their serum proteins; (*ii*) that when treated with NSAIDs, peptide binding to these patients' serum proteins is reduced; (*iii*) that patients with jaundice or who are pregnant (and have natural remission of their arthritis) also had fewer bound peptides, and (*iv*) the finding that only NSAIDs (including gold and chloroquine) but not other acidic drugs (phenobarbital, paracetamol, penicillins) displace tryptophane and certain peptides (containing Try or PhA1) from protein binding sites in human serum and bovine albumin (138, 139). The essence of this thesis is that "in patients with rheumatoid arthritis the anti-inflammatory peptides are bound to an abnormal extent to the circulating proteins, and the anti-arthritic drugs act by redressing the bound: free ratio to that in the normal subject (14)."

Albumin binding has been invoked as a mechanism contributing to the uricosuric activity of many acidic NSAIDs, effectively displacing protein-bound urate in vivo (140). The capacity of whole serum to bind urate, studied in vitro, is reduced after the in vivo ingestion of phenylbutazone, mefenemic acid, and salicylates (141). In vitro studies of urate displacement from HSA have indicated that a wide range of acidic drugs share this property (120, 142); alcohols corresponding to some of the active acids (e.g. flufenamic, Ibuprofen), though binding to the albumin, were devoid of urate-displacing activity. These in vitro studies indicated that only acids attaining blood levels of 10 μg/ml plasma could be expected to induce significant uricosuria, through displacement of albumin-bound urate (though still perhaps promoting urate excretion through other renal mechanisms, e.g. stimulating tubular secretion).

Two points are worth emphasizing in connection with these "albumin-binding" theories of NSAIDs action.

A. Drugs would be "active" only when albumin-bound, i.e. having thereby displaced tryptophane, urate, etc.; in contrast to the usual pharmacological dogma that only the unbound drug is active. Indeed, Smith & Dawkins (14) have stated that it is only the unbound salicylate that is toxic.

B. Such in vitro methodologies may be of real value for recognizing active drug metabolites (120, 143), especially those formed from drug precursors that are themselves inactive in vitro but effective when administered in vivo.

The contention that NSAIDs displace corticosterone from its binding sites on rat plasma proteins (144) has been hotly disputed, mainly on the basis of studies of the normal binding of 11-hydroxycorticosteroids to human plasma in the presence of various NSAIDs (145–148) or the failure of these drugs to lower corticosterone levels in rats (149). The general consensus is that NSAIDs do not owe their in vivo activity to displacement of (otherwise inactive) anti-inflammatory steroids from silent-receptors on plasma proteins.

In human serum, L-tryptophane is the only amino acid bound to protein: its ready displacement by salicylate in vitro (150) and after ingestion of aspirin (151) may account for the abnormally high urinary excretion of certain tryptophane metabolites by patients with rheumatoid arthritis.

The hypoalbuminemia of rheumatoid patients, especially in the later stages of the disease (152–155) and in adjuvant arthritic rats (155–158) should be considered in assessing novel acidic NSAIDs; reduced binding in the arthritic subjects, compared to the reference population, may be manifest in difficulties in tolerating the drug due to higher levels of the unbound drug anion.

Gastric Toxicity and Ulcer Formation

Epidemiological evidence for the association of aspirin ingestion and acute gastrointestinal (GI) bleeding has been reviewed (159). Many studies demonstrated that an unduly high proportion of patients hospitalized with acute upper GI hemorrhage had taken aspirin-containing analgesics. However, because of deficiencies in experimental design and suitability of control subjects, there was no unequivocal proof that aspirin played a causal role. An excess of gastric ulcers in Australian women was associated with consumption of large amounts of aspirin-containing headache powders (160, 161). Animal studies showed that aspirin, given orally, decreased healing of gastric ulcers in dogs (162). Increased occult fecal blood loss during salicylate administration is well documented, but the relationship of occult bleeding and severe hemorrhage is not clear (159). Ethanol ingestion may increase the occult blood loss induced by aspirin (163, 164). Occult blood loss can be reduced or prevented by administering aspirin in a solution of sufficient buffer capacity to reduce appreciably the acidity of the gastric juice (165). The important role of acid in the gastric irritation produced by aspirin is confirmed by studies in achlorhydric subjects who had fewer mucosal erosions and less occult blood loss than normal subjects given the same aspirin dose (166–168). However, even in achlorhydric subjects, occult blood loss increased three or four-fold when aspirin was ingested (166, 167). Although intravenous aspirin prolongs the bleeding time to the same extent as oral aspirin, it does not increase blood loss in human subjects (169, 170). These studies suggest that the toxic effects are predominantly local and are supported by animal studies (171, 172) which were unable to relate gastric toxicity to plasma salicylate concentration.

Aspirin "breaks" the normal gastric mucosal barrier against back diffusion of hydrogen ions, which then may injure the submucosal capillaries, with subsequent necrosis and bleeding (173-175). This increased permeability has been confirmed by studies of changes in potential difference (176) and in diffusion of lithium ions across the gastric mucosa (177). The gastric mucus layer of rats was decreased by calcium acetylsalicylate, oxyphenbutazone, and indomethacin (178). Mucin extracts prepared from pig gastric mucus demonstrated in vitro aggregation of a 4-S fraction by salicylate at pH 3.6, but not at pH 7.3 (179), suggesting that impairment of the mucosal barrier may be caused by precipitation of a protective glycoprotein component of gastric mucus by salicylate. Paradoxically, acid secre-

tion in vitro by pig gastric mucosa is decreased by 1.5 mM, and completely prevented by 3 mM sodium salicylate (180).

These findings were not confirmed in studies of human gastric juice (181). However, "KL-11", a lysine derivative that increases mucopolysaccharide biosynthesis, inhibits the development of gastric ulcers following ligation of the pylorus in rats (182), suggesting that if mucin production could be increased, mucosal damage by anti-inflammatory drugs would be prevented. Exposure to solutions of aspirin increased gastric mucosal blood flow of dogs as measured by the gastric mucosal clearance of aminopyrine (183). In addition, the leakage of plasma across the rat gastric mucosa (measured by Pontamine Sky Blue) was increased by mucosal contact with solutions of aspirin, but decreased when aspirin was administered intraperitoneally or intraduodenally (184).

Damage to mouse gastric mucosal cells exposed to 20 mM solutions of aspirin began within one minute of contact, and within ten minutes many surface cells were lysed, producing superficial erosions and bleeding; but eight hours later no lesions could be found (185). The rate of exfoliation of dog gastric mucosal cells was increased by oral aspirin or phenylbutazone (186), while the mitotic activity of rat gastric mucosa (measured by uptake of thymidine-^{3}H (186) or by the mitotic index (187) was decreased by subcutaneous administration of these NSAIDs. Indomethacin given orally to dogs with isolated antral fistulas caused ulceration of the isolated antrum and a decrease in the volume of antral secretion, but no decrease in mucus content or parietal cell population (188).

With the present availability of many acceptable methods for evaluating the gastric toxicity of NSAIDs in both animals and man, the search for new non-irritating NSAIDs should be greatly facilitated.

New Compounds with Anti-Inflammatory Properties

Disclosures in the nonpatent literature since the last review (1) continue to emphasize the carrageenin-induced rat paw edema as the prime criterion for judging a compound to be a NSAID. The shortcomings of this approach are indicated by recent reports that cycloleucine (189), triethylphosphine aurochloride (190), and clofazimine (Lamprene®) (191) inhibit the rat adjuvant arthritis but do not inhibit acute edemic inflammation.

New acidic NSAIDs include arylalkanoic acids (192–209), tetrazoles (210–212), sulfonamides (213, 214), diones (215–224), hydroxamates (225, 226), ortho-substituted benzoates (227–229), and other acids (230–232). Among the various bases disclosed (233–248), only flazalone (R-760) (249, 250) seems to have been accepted into the clinic. Other new agents effective in animals include pyrazoles (251–255), indoles (256), bi- and tri-cyclic heterocyclic compounds (257–266), various natural products (267–274), inorganic materials (275, 276), and miscellaneous organic compounds (277–282); though few could be considered to approach the steroids or indomethacin in potency.

The sad history of fenclozic acid (283), is an illustration of how a drug effective in the clinic may still have to be withdrawn on account of an unpredictable toxicity (jaundice), despite the most thorough toxicity testing in animals.

ANTI-INFLAMMATORY PROTEINS AND POLYPEPTIDES

Doubt has been cast on the accepted structure for human ACTH (284): the proposed revised structure closely resembles that of porcine ACTH with the sole substitution of serine (human) for leucine (porcine) at position 31.

Anti-inflammatory proteins (AIP) have been studied with renewed interest. Endogenous AIP are present in inflammatory exudates, and to a lesser extent in serum, after injecting both soluble and insoluble irritants (285–290). While some of the AIP may arise from local tissue or leukocytic necrosis, definitive evidence has been obtained that an AIP is synthesized by the liver in response to extrahepatic inflammation (291). When injected into virgin animals these AIP preparations inhibit foot paw edema elicited by carrageenin, kaolin, etc. and even the adjuvant arthritis (291), possibly by acting as counter-irritants (290, 292).

Some of the anti-inflammatory activity in preparations of antilymphocyte sera (ALS) (293–295) is probably due to AIP formation following an inflammatory response by the immunized host to repeated i.v. injection of heterologous lymphocytes. However, even purified ALS preparations may display anti-inflammatory activity per se by depleting an animal of complement, a pro-inflammatory agent (296), as a consequence of combining with the lymphocytic antigen(s).

High levels of pancreatic proteases, bromelain and ficin (but not papain) administered orally inhibit edemas elicited by a variety of inflammagens (297–299). Porcine pancreatic proteases given orally to surgical patients accelerated the decline in serum α_1-antitrypsin activity toward normal levels following surgery (300) when compared with placebo. It is possible that these clinically effective enzyme preparations elicit the well-known counter irritant phenomenon, which can depress acute or traumatic inflammation (301), without themselves being "irritants" in the usual sense.

Proteins that inhibit proteases also control certain types of clinical inflammation: unfortunately the soybean protease inhibitor and ovomucoid induce allergic and inflammatory responses to themselves, precluding their use in man. Trasylol (a low mol wt inhibitor of bovine origin) used for pancreatitis (302, 303) also inhibits leukocyte proteases (304).

FINAL COMMENT

Almost by definition, NSAIDs only moderate the intensity of the signs and symptoms of inflammation, but do not eradicate the underlying disease, or even significantly change its course. Whenever the etiology of an inflammatory condition is discovered, and specific anti-etiologic therapy is developed, treatment with NSAIDs becomes irrelevant. Such developments imply the ultimate obsolescence of the currently available NSAIDs unless perhaps it should be proved that chloroquine and gold preparations are indeed anti-etiologic (e.g. Mycoplasmastatic) agents.

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