CHAPTER 1

ANTI-INFLAMMATORY DRUGS IN THE 21ST CENTURY

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Abstract: Historically, anti-inflammatory drugs had their origins in the serendipitous discovery of certain plants and their extracts being applied for the relief of pain, fever and inflammation. When salicylates were discovered in the mid-19th century to be the active components of Willow Spp., this enabled these compounds to be synthesized and from this, acetyl-salicylic acid or Aspirin™ was developed. Likewise, the chemical advances of the 19th–20th centuries lead to development of the non-steroidal anti-inflammatory drugs (NSAIDs), most of which were initially organic acids, but later non-acidic

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compounds were discovered. There were two periods of NSAID drug discovery post-World War 2, the period up to the 1970’s which was the pre-prostaglandin period and thereafter up to the latter part of the last century in which their effects on prostaglandin production formed part of the screening in the drug-discovery process. Those drugs developed up to the 1980-late 90’s were largely discovered empirically following screening for anti-inflammatory, analgesic and antipyretic activities in laboratory animal models. Some were successfully developed that showed low incidence of gastro-intestinal (GI) side effects (the principal adverse reaction seen with NSAIDs) than seen with their predecessors (e.g. aspirin, indomethacin, phenylbutazone); the GI reactions being detected and screened out in animal assays. In the 1990’s an important discovery was made from elegant molecular and cellular biological studies that there are two cyclo-oxygenase (COX) enzyme systems controlling the production of prostanoids [prostaglandins (PGs) and thromboxane (TxA2)]; COX-1 that produces PGs and TxA2 that regulate gastrointestinal, renal, vascular and other physiological functions, and COX-2 that regulates production of PGs involved in inflammation, pain and fever. The stage was set in the 1990’s for the discovery and development of drugs to selectively control COX-2 and spare the COX-1 that is central to physiological processes whose inhibition was considered a major factor in development of adverse reactions, including those in the GI tract. At the turn of this century, there was enormous commercial development following the introduction of two new highly selective COX-2 inhibitors, known as coxibs (celecoxib and rofecoxib) which were claimed to have low GI side effects. While found to have fulfilled these aims in part, an alarming turn of events took place in the late 2004 period when rofecoxib was withdrawn worldwide because of serious cardiovascular events and other coxibs were subsequently suspected to have this adverse reaction, although to a varying degree. Major efforts are currently underway to discover why cardiovascular reactions took place with coxibs, identify safer coxibs, as well as elucidate the roles of COX-2 and COX-1 in cardiovascular diseases and stroke in the hope that there may be some basis for developing newer agents (e.g. nitric oxide-donating NSAIDs) to control these conditions.

The discovery of the COX isoforms led to establishing their importance in many non-arthritic or non-pain states where there is an inflammatory component to pathogenesis, including cancer, Alzheimer’s and other neurodegenerative diseases. The applications of NSAIDs and the coxibs in the prevention and treatment of these conditions as well as aspirin and other analogues in the prevention of thrombo-embolic diseases now constitute one of the major therapeutic developments of the this century. Moreover, new anti-inflammatory drugs are being discovered and developed based on their effects on signal transduction and as anti-cytokine agents and these drugs are now being heralded as the new therapies to control those diseases where cytokines and other non-prostaglandin components of chronic inflammatory and neurodegenerative diseases are manifest. To a lesser extent safer application of corticosteroids and the applications of novel drug delivery systems for use with these drugs as well as with NSAIDs also represent newer technological developments of the 21st century. What started out as drugs to control inflammation, pain and fever in the last two centuries now has exploded to reveal an enormous range and type of anti-inflammatory agents and discovery of new therapeutic targets to treat a whole range of conditions that were never hitherto envisaged.

1. HISTORICAL DEVELOPMENTS

The anti-inflammatory analgesic drugs have their origins in the use of extracts of salicylate-containing plants, especially the bark of the willow tree (Salix alba and other members of the Salix species), in the treatment of fever, pain and inflammatory
Anti-inflammatory drugs in the 21st century

conditions (Rainsford, 2004a). These treatments date from early Chinese, Indian, African and American eras and were initially described in some detail by Roman and Greek medical authorities. During the 17th–19th centuries, the popularity of these plant extracts became evident following the publication by the Reverend Edward Stone in the 17th century of probably what were the first clinical trials of willow bark extract for the treatment of agues or fever. Isolation of the principally-active salicylate components followed in the early 19th century and with advances in chemistry in Europe and developments in the German chemical industry in the mid-late 19th century, there followed the synthesis or salicylic and acetylsalicylic acids, the latter being highly successfully commercialised by Bayer AG as Aspirin™ over 100 years ago. The historical aspects of the origins and development of aspirin and other salicylates are told in detail elsewhere (Rainsford, 2004a). During the period of the exploitation of the by-products of the coal tar industry in Germany in the 19th century came also the development of antipyretic/analgesic agents, antipyrine, aminopyrine, phenacetin and later following recognition of paracetamol (acetaminophen) as the active metabolite of phenacetin, this was eventually commercially developed for use as an analgesic/antipyretic agent in the 1950’s (Prescott, 2001).

1.1. Discovery of NSAIDs

The development of the first of the category of what are now known as the non-steroidal anti-inflammatory drugs (NSAIDs) of which aspirin has now become recognised as the progenitor, was phenylbutazone in 1946 (by JR Geigy, Basel, Switzerland) and later indomethacin in the 1960’s (by Merck & Co, Rahway, NJ, USA) (Otterness, 1995). Phenylbutazone was initially employed as a combination with antipyrine in the belief it would enhance the actions of the latter. However, it emerged to have greater anti-inflammatory/analgesic activity than antipyrine and was for the best part of 30 years successfully used for the treatment of arthritic and other painful inflammatory conditions until its popularity progressively waned after associations with life-threatening agranulocytosis and bone marrow suppression (still essentially not conclusively proven today), upper gastrointestinal ulcers and bleeding and subsequent popularity of more advanced NSAIDs.

Ibuprofen was developed by Boots (UK) in the 1950–1960’s and after establishing its favourable safety profile at dose ranges for analgesic and anti-pyretic efficacy (up to 1200mg daily) it was the first NSAID (other than aspirin) to be approved for non-prescription (over-the-counter or OTC sale) use in the UK (in 1963), then the USA (in 1964) and later in many other countries worldwide (Rainsford, 1999). Just after ibuprofen was developed, a large number of pharmaceutical companies undertook the discovery and development of NSAIDs with a range of chemical and biological properties (Evans & Williamson, 1987; Otterness, 1995; Rainsford, 1999, 2004a, 2005a). The general chemical categorization of these drug classes are shown in Figure 1. Most of these drugs developed in the 1960’s were discovered in the pre-prostaglandin era (i.e. before Vane and his
Figure 1. Chemical Classification of the NSAIDs

colleagues had discovered the inhibitory actions of aspirin and related drugs on the production of prostaglandins). Their anti-inflammatory, analgesic and anti-pyretic properties were discovered using animal models with some supportive properties being established in some biochemical systems which were known also to be important in inflammation (e.g. mitochondrial oxidative, intermediary and connective tissue collagen and proteoglycan metabolism; stability of albumin; and later oxyradicals).

2. COX-2 SELECTIVE AGENTS AND THE COXIBS

Coxibs belong to a class of nonsteroidal anti-inflammatory drugs (NSAIDs) that are used to treat pain and inflammation in a variety of acute and chronic conditions. They have been principally employed for treating rheumatoid and osteo-arthritis, and other arthritic diseases, dental and surgical pain in post-operative settings, dysmenorrhoea, and acute injuries (Kean & Buchanan 2005). The coxibs have
also been explored for the prevention of colorectal and some other cancers (Harris, 2002a; see later section) as well as Alzheimer’s disease (Firuzo & Practico, 2006), although the outcomes of these studies have not been particularly favourable largely through lack of efficacy and/or cardiovascular complications. Indeed, the apparent high risk of myocardial infarctions and the exacerbation of symptoms of hypertension and elevation of blood pressure led to the worldwide dramatic withdrawal of one of the leading members of the coxib class, rofecoxib, by the Merck Company on September 29, 2004 (Rainsford, 2005b). This has been followed by the recommendation of the US Food and Drug Administration in April 2005 that Pfizer Inc, the company manufacturing other leading coxibs (celecoxib and valdecoxib) also withdraw valdecoxib from the US market because of the same adverse events. Questions have now been posed whether a cardio-renal syndrome is associated with the entire class of coxibs – a class effect – that may account for the mortality or non-fatal myocardial infarctions and elevation of blood pressure associated with these drugs, possibly in at risk subjects (as yet undetermined). The US FDA has subsequently specified a black box warning on the use of celecoxib and all other coxibs (that remain on the market or in clinical trial) and also a general warning of cardiovascular risk with all other NSAIDs. The European Medicines Evaluation Agency (EMEA), now the European Medicines Agency (EMA) has also re-evaluated the cardiovascular risk with the coxibs and has recommended only restricted use of these drugs. Thus, in somewhat over half a decade since their much-heralded introduction as being safer to the gastrointestinal tract and kidneys than traditional NSAIDs and with rofecoxib and celecoxib having achieved worldwide market domination, they have now plummeted from sales to almost obscurity in the therapeutic armamentarium. There are indications, however, that celecoxib may find its way back onto the world markets but the future of rofecoxib is less certain and maybe it will find applications (e.g. in juvenile rheumatoid arthritis in which it was especially effective) but under very strictly restricted conditions. Another Merck drug, etoricoxib, might not be associated with an excess risk of cardio-renal effects and associated myocardial infarction and exacerbation of hypertension. Likewise, although less adequate data are available with lumiracoxib, there are suggestions this drug may not have the same risks as seen with rofecoxib or other coxibs.

A key factor that has emerged from the analysis of reasons why rofecoxib, valdecoxib, and celecoxib may have led to development of the cardio-renal syndrome thought to underlie myocardial infarction and hypertension appears to have been that these effects were apparent with high dose levels of these drugs. It is possible that in some of the conditions where they were being used (e.g. a colorectal preventative trials of rofecoxib and celecoxib and post-operative coronary bypass in the case of valdecoxib) may have been conditions where there were appreciable manifestations of disease stress that led to pre-disposition to the development of cardio-renal syndromes and myocardial infarction. A major factor was dosage and the data indicates that the cardio-renal syndrome and cardiovascular risks were only evident with high doses of these drugs. Another factor which has emerged is
that the principal mode of action of these drugs, to specifically inhibit the enzyme, cyclooxygenase-2 (COX-2) may have been a major factor causing the development of these site effects – this being an example of what is known as “mechanism-based” toxicology.

Coxibs are strictly classed as *functional analogues* since aside from the general chemical features in common with members of this class there are few common specific chemical features that uniformly describe their properties. There are, of course, some features of the biochemical interactions of these with the enzyme, COX-2, which mediates their main pharmacological actions. With the possibly unique exception of lumiracoxib, the other coxibs are tricyclic compounds with high pKa values (pKa 8–9). These contrast with the conventional NSAIDs that are weakly acidic compounds with pKa values of about 3–5, derived from either aryl-carboxylic acids or keto-enolic compounds. The coxibs are diaryl-heterocycles that have a *cis*-stilbene moiety substituted in one of the pendant phenyl rings with a 4-methylsulphone (e.g. rofecoxib) or sulfonamide (e.g. celecoxib) substituent (Figure 2). These moieties are critical together with the diaryl heterocyclic structure in determining their actions as highly specific COX-2 inhibitors.

The odd drug apparent in these chemical associations within the coxibs is lumiracoxib. This drug is an analogue of the traditional acid NSAID, diclofenac, and does not have the tricyclic character of the other coxibs but is an anilino-phenylacetic acid. The 2,6-dichloro-substituents of diclofenac are replaced by 2-chloro, 6-fluoromoieties in lumiracoxib. There are indications that the COX-2 specificity of lumiracoxib. Perhaps this drug should not be classed as a coxib in view of the lack of associations both chemically and possibly pharmacologically with the other coxibs.

The term coxib derives logically from *cox-inhibitor* and appears to have been a marketing ploy by the two major companies that developed these drugs to discriminate them from other NSAIDs. Whether such a pharmacological description is
justifiable is debatable especially since the claims for markedly improved GI safety with the coxibs are now being increasingly challenged in relation to at least the risk of serious GI adverse reactions observed with low-risk NSAIDs such as diclofenac or ibuprofen.

2.1. Rationale for the Discovery of Coxibs

The discovery in 1991 of two COX enzymes that are responsible for the synthesis of inflammatory prostaglandins gave a new basis for understanding how these molecules regulate and mediate inflammatory reactions, pain and fever, as well as a number of diverse physiological reactions such as blood flow, thrombosis, and gastrointestinal, renal and reproductive functions (Rainsford, 2004e). About two years previously, a unique COX enzyme was discovered that was produced in response to inflammatory stimuli. In a short while, the genes coding for two separate enzyme proteins were isolated and cloned. By convention the enzyme that is responsible for the production of physiologically important prostaglandins and thromboxane \(A_2\) is termed COX-1. The other enzyme, which is responsible for prostaglandins involved in inflammation and pain and is induced upon stimulation with various inflammatory stimuli (lipopolysaccharide, growth factors etc.), is known as COX-2. Actually the term COX refers to the cyclooxygenase enzyme activity and since peroxidase activity is also present, both enzymatic properties exist in one protein which is termed prostaglandin G/H endoperoxide synthase or PGHS. COX-1 is present in PGHS-1 and COX-2 in PGHS-2. Because the enzymatic activity is the functional response to the gene-regulated and expressed production of prostanoids (prostaglandins and thromboxane \(A_2\)) it is usual to term the two isoforms COX-1 and COX-2 for short.

2.1.1. Prototypes of the coxibs

Two classes of COX-2 selective inhibitors have, in retrospect, emerged as the prototypes for the development of the coxibs. These are (a) a group of aryl sulphonanilides typified by NS298, nimesulide (R-805), flosulide (CGP28238), diflumidone (R-807), T-614, L-745,337 and FR115068, and (b) the 1,2-diarylheterocyles, DuP697 and SC58125 (De Leval et al., 2000; Rainsford, 2004e). Thus, many COX-2 selective agents have been discovered by taking the sulphonanilide moiety and superimposing this on various diaryl heterocycles. The sulphonanilide could be a 4-methylsulfonyl- or sulphonamide in one of the pendant phenyl rings; the former being attached to a cis-stilbene moiety. It has been suggested that the origins of the diaryl-substituted heterocycles are from phenylbutazone, which led to the development of indoxole and oxaprozin. Indoxole was then considered to have been the precursor of DuP697 and SC58125. Some have claimed that indomethacin may have served as a basis for development of diaryl heterocycles with the acetic acid moiety being modified to be replaced by a sulphonanilide. Probably the first sulphonanilide to be developed which has emerged as a clinically successful COX-2 selective drug was nimesulide. This drug was initially discovered
(coded R-805) by Riker in the 1960’s as part of a programme to identify anti-inflammatory analgesic drugs based on sulphonamides [Rainsford, 2005a]. Clearly, these studies took place and this drug developed as a clinically effective drug some three decades before the COX-isoforms were discovered. Screening for relative COX-1/COX-2 activities of established NSAIDS was initially undertaken by a number of academic groups as well as in the pharma industry after the COX-isoforms were discovered in the early 1990’s. Thus, nimesulide emerged from these studies and as well, meloxicam, a derivative of piroxicam was also found to have COX-2 selectivity [Trummlitz & van Ryn, 2002]. Meloxicam is different from other COX-2 selective drugs in being an enolcarboxamide although structural studies with COX-isoforms investigated by molecular modelling has confirmed its fit with the active site of COX-2 [Trummlitz & van Ryn, 2002].

The development of nimesulide was predicated on the search for anti-oxidant compounds. The carboxyl group of NSAIDs (then regarded as a prerequisite for anti-inflammatory activity of aromatic drugs, the NSAIDs) was replaced by nitro- and sulphonamides to give putative anti-oxidant compounds with higher pKa values (6.5–7.0) than conventional carboxylates (pKa 2.5–4.0) or keto-enolates (e.g. phenylbutazone) (pKa 4.5–5.5).

In summary, the identification of nimesulide and meloxicam, along with etodolac and oxaprozin as agents having COX-2 selectivity, has emerged long after these drugs were introduced clinically. The search for highly selective COX-2 inhibitors proceeded on the basis that more potent and selective inhibitors of COX-2 would be more effective in controlling pain, inflammation and fever, and with fewer side effects in the gastrointestinal tract and possibly the kidneys than the above mentioned established COX-2 selective NSAIDs as well as others of this class of drugs.

2.1.2. Development of the coxibs

The chemical development of the coxibs has been comprehensively reviewed by a number of authors, to whom the reader is referred for more detailed information [Dannhardt & Laufer, 2000; De Leval et al., 2000]. Here, some of the salient features of the development of the coxibs are outlined [Rainsford, 2004c]. The basis for the identification of COX-2 selectively has been the development of in vitro assays. These have comprised (a) isolated recombinant enzymes, (b) cell lines with COX-2 and COX-1 activity, (c) primary cells e.g. platelets or platelet rich plasma as a source of COX-1 and stimulated macrophages for COX-2 activities respectively, (d) cell lines (e.g. chinese hamster ovary, (CHO) cells transfected with either human recombinant COX-2 or COX-1 genes, and (e) variants of whole blood assays in which COX-1 activity is determined after 1 hr by measuring thromboxane production, and COX-2 after incubation with lipopolysaccharide (endotoxin of Escherichia coli) or interleukin-1 for 24 hr and measuring prostaglandin E2 production. Each of these assays has its merits and applications. Mostly, assays (a) and (d) were employed in the discovery of relative COX-2/COX-1 activity. The whole blood assay has
been regarded as more appropriate for determining the clinically relevant COX-2 selectivity especially in relation to the plasma pharmacokinetics of the drugs.

In determining the structural requirements for COX-2 selectivity, drug modelling of interaction with the COX-isoenzymes has been made possible because of the availability of crystal structures of the ovine COX-1, murine COX-2 and human COX-2, which have been solved to 3 to 3.5 Å resolution (Trummlitz & van Ryn, 2002).

3. NOVEL NSAIDs AND DERIVATIVES

Over the century or more since the discovery of drugs used to treat inflammation, pain and fever there have been an immense number and variety of chemical analogues and derivatives that have been developed some of which have found successful application in treating inflammatory diseases and some have passed out of favour or use for one reason or another (Adams & Cobb, 1967; Otterness, 1995; Rainsford, 1999a, 1999b, 2004a, 2004b, 2004c, 2004d, 2005a). Many of the older agents could usefully be employed given understanding that they may have unique modes of action in controlling different pathways or cellular reactions in inflammatory diseases. Their potential for exploitation is phenomenal!

3.1. Nitric Oxide – Donating NSAIDs

The development of nitric oxide (NO) – donating NSAIDs had its origin in the recognition that nitric oxide has an important role in regulating blood flow and vascular functions and that NO donors could protect the gastro-intestinal (GI) mucosa against injury by NSAIDs and various necrotizing agents (Whittle, 2003). The idea of chemically coupling an NO-donor to an NSAID in the form of an acidic ester, such that upon absorption by the gastric mucosa NO would be released to produce local vasodilatatory effects and protection of the mucosa from injury from the NSAID seemed an elegant means of developing NSAID derivatives that would be notably safer to the GI tract than the parent NSAID. It has been known for over 40 years that simple alkyl or phenyl esters of NSAIDs have less gastro-irritancy than their parent acids (Rainsford, 2004b, 2004f) so that esterification with NO-donating groups would also be expected to confer protection against the injurious effects of the acidic moieties of NSAIDs. To what extent the addition of an NO-donor to the alkyl or other ester adds to the protective effects has not been determined. While much work has been done to establish the actions of different NO-NSAIDs (Keeble & Moore, 2002; Whittle, 2003; Zacharowski et al., 2004; Corazzi et al., 2003, 2005; Dhawan et al., 2005) and many derivatives have been developed (Chiroli et al., 2003; Whittle, 2003; Gao et al., 2005) to date this has not yet clearly translated into clinically useful drugs although many hold promise. The development of nitro-aspirin for prevention of cardiovascular disease (Whittle, 2003; Abrosini et al., 2005) must hold the greatest promise as present. There are, however, exciting prospects for exploiting the pro-apoptotic effects of NO-NSAIDs (Huguenin et al., 2004a, 2004b; Royle et al., 2004; Fabbri et al., 2005; Bolla & Zoli,
pro-oxidant effects (Gao et al., 2005) and inhibition of MAP kinase pathways (Hundley & Rigas, 2006) in the prevention and treatment of a variety of different cancers.

3.2. Resolvins or Epilipoxins

These products of lipoxygenase (LOX) activities, among them the aspirin-triggered lipoxin (Rainsford, 2004c; Serhan, 2005) and the products of omega-3 fatty acid metabolism through the LOX pathways, and stable analogues thereof that have been found to have anti-inflammatory activity now attract much interest as potential therapies not only for treating inflammatory diseases but also the inflammatory components of cancers and many other chronic diseases (Serhan et al., 2004; Petasis et al., 2005; Serhan, 2005; Parkinson, 2006). These fatty acid derivatives hold particular promise because of their structural novelty and unique lipoxin receptor targets.

3.3. COX-3 as a Therapeutic Target?

There has been much speculation and interest in the possibility that there may be another cyclooxygenase in addition to COX-1 or COX-2 which could be a target for actions of analgesics e.g. paracetamol (acetaminophen) (Berenbaum, 2004; Lucas et al., 2005). Despite earlier discovery of a variant of COX-1 in some regions of the brain and suggestions that paracetamol may act selectively on this variant, it is now clear from recent experimental studies and evaluation of the earlier evidence that COX-3 is actually a splice variant of COX-1 (Figure 3) and that acetaminophen

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**Figure 3.** Structure of the genome for cyclooxygenase-3 contrasted with that of COX-1, showing the splice variant nature of COX-3 and its derivation by inclusion on intron-1. Based on information in Berenbaum (2004)
while inhibiting cyclooxygenase activity in intact cells (but not in broken cell preparations) does so by interfering with the oxidant status of cells (Lucas et al., 2005) in a manner that resembles the anti-oxidant actions of phenolic compounds. Thus the search for analgesics that might act on the suggested COX-3 enzyme would appear to be an exercise in futility.

4. DISEASE-MODIFYING AGENTS & CORTICOSTEROIDS

The term “disease-modifying” agent (involving use of disease-modifying anti-rheumatic drugs, DMARDs) has had particular vogue in the therapy of rheumatic diseases where attempts to achieve control or at best reversal of the chronic inflammatory disease have involved a variety of approaches (Paulus, 1995; Abadie et al., 2004; Simon, 2004). For treatment of rheumatoid arthritis, a whole range of drugs have been employed over the years. Among these are the parenteral and oral gold salts (whose use has been given the term “crysotherapy”) and D-penicillamine, whose uses were discovered serendipitously as well as chloroquine or hydroxychloroquine, sulphasalazine, cyclosporine A, levasimo, azathioprine, cyclophosphamide, chlorambucil and methotrexate (Brooks et al., 1985; Simon, 2004) as well as combinations of some of these drugs in therapy resembling the approaches employed in cancer therapy (Paulus, 1995). Of these agents, probably methotrexate has now assumed greatest use as a first line therapy having relatively fewer severe adverse reactions, some of which are manageable, compared with many other DMARDs. Likewise, corticosteroids have had considerable popularity since the discovery by Hench at the Mayo Clinic in 1949 that cortisone had a dramatic effect in bed-ridden patients suffering with severe rheumatoid arthritis (Evans & Williamson, 1987; Hirschmann, 1992). Indeed the synthetic developments and large commercial efforts put into the discovery of corticosteroids (or glucocorticoids) made in the 1950s–1980’s (Evans & Williamson, 1987; Hirschmann, 1992; Berstein, 1992) only to yield the relatively few drugs used today is a striking reflection of the complex nature of this class of compounds. Indeed, it was originally considered that corticosteroids acted by immunosuppressive activity, an idea arising due to the effects that occurred at the high doses that were given in former times (Brooks et al., 1985). Long after their discovery, advances in the molecular biology of inflammatory mediator production have shown that these drugs act principally through their binding to the specific glucocorticoid receptor (GR*) and consequent inhibition of cellular signalling pathways (especially AP-1 and NFκB) that regulate the production of inflammatory cytokines and chemokines (IL-1, TNF-α, IL-2, IL-5, IFN-γ, GM-CSF, MIP-1, MCP-1, Eotaxin, RANTES), cell adhesion molecules (I-CAM, V-CAM, E-selectin), COX-2, cPLA_2 and iNOS that control production of eicosanoids and NO, and the production of joint-destructive metalloproteinases (Vayssiere et al., 1997; Russo-Marie, 2004). The inhibitory effects of corticosteroids on production of acute phase proteins and erythrocyte sedimentation rate (ESR) are indicators of the effectiveness of these drugs on pathognomonic biomarkers of chronic disease (Russo-Marie, 2004). In some respects the corticosteroids are ideal
anti-inflammatory drugs for use in chronic disease but their side-effects are proven formidable and often severe or irreversible (e.g. bone damage, immunosuppression and propensity to infection, gastrointestinal ulcers and bleeding, skin thinning) and in part their undoing. While low-dose corticosteroids are now used commonly in rheumatoid arthritis, concerns about their long-term use are still a matter of major concern.

In the past two decades, significant advances have been made in the molecular biology of the cells and mediators of chronic inflammatory disease and the biotechnological processes involved in the production of peptides, anti-bodies (including “humanized” monoclonal anti-bodies, or hMAbs, from mouse precursors) and the isolation and cloning of genes regulating the production of endogenous inhibitors or soluble receptors that interact with pro-inflammatory cytokines as well as the production of anti-inflammatory cytokines. These developments have led to a revolution in the therapy of not only rheumatoid arthritis, but also chronic inflammatory diseases affecting a large number of organ systems (e.g. ulcerative colitis, Crohn’s disease, psoriasis) [Katz, 2005]. Of the principal anti-cytokine biological therapies that have been developed and are now given parenterally in treating rheumatoid arthritis in select patients, the following drugs have specific targets, namely:

1. **Target: TNFα**
   - Etanercept – Recombinant TNF-R Fc fusion protein.

2. **Target: IL-1**
   - Anakinra – recombinant human IL-1Ra protein.

In rheumatoid arthritis, all except infliximab are used as monotherapies; otherwise, drugs are usually given with methotrexate. The outcomes from therapy with these agents can be summarized as follows (see Weaver, 2004; Crum et al., 2005).

1. Anakinra used alone or with methotrexate reduces clinical signs and symptoms of RA.
2. TNF inhibitors show similar efficacy and have higher response rates for clinical and radiological parameters than with anakinra.
3. There is a question of whether long-term therapy produces radiological evidence of reduced joint disease.
4. There are major issues about infections due to immunosuppression.

The applications of these biological therapies have been considered with much caution following the initial concerns about immunosuppression and consequent predisposition to conditions such as latent tuberculosis. In many countries there are now patient registries for rheumatic patients receiving these biological therapies and this reflects the need for careful therapy and monitoring. What has been learnt from their application is, however, that the respective pro-inflammatory cytokine targets have valid “proof of concept” for their importance in treating rheumatoid arthritis, and where these therapies have also been found effective in other conditions there is also support for the central concept of controlling pro-inflammatory cytokines in these chronic inflammatory diseases.
5. ANTI-CYTOKINE AGENTS AND SIGNAL TRANSDUCTION INHIBITORS

In the light of the above conclusions about the importance of pro-inflammatory cytokines in chronic disease, it is not surprising that the emphasis in recent years has been to develop small molecules to target the processes governing the control of their actions. Several NSAIDs affect the production or actions of cytokines and this property has been considered to be a component of their actions, positive or negative. Thus, indomethacin and some other NSAIDs may increase production of interleukin-1 (IL-1 or tumour necrosis factor-α (TNF-α) and these effects have been considered important in the development of GI ulcers and asthma attributed to these drugs. However, some other NSAIDs such as nimesulide inhibit IL-6 and TNF-α (Rainsford et al., 2005) while ibuprofen inhibits TNF-α (Jiang et al., 1998). TNF-α induction of the NFκB/IkB signalling pathway is inhibited by salicylate at the level of the activity of IKKinase and cAMP-response element binding protein (CREB) (Rainsford, 2004b). These effects are considered among the anti-inflammatory effects of these drugs. The inhibitory effects on signalling pathways, especially those involving NFκB/IkB and MAP kinases, may have particular significance in subsequent inhibition of the expression of mRNAs and the proteins of COX-2, iNOS and PLA2 (Rainsford, 2004; Rainsford et al., 2005b). With nimesulide there is also an interesting additional property that this drug activates glucocorticoid receptors leading to down-regulation of a number of cytokines, metalloproteinase enzymes, COX-2, iNOS and PLA2 (Rainsford et al., 2005b). Some NSAIDs also affect the response of T-cells to IL-2 (Hall et al., 1997) and this together with reduction in the effects of PGE2, due to blockade of the production of this prostanoid by NSAIDs, may form a component of their immuno-regulatory effects (Smith et al., 1971; Goodwin et al., 1977, 1978).

The original observations of the inhibitory effects on NFκB activation by high concentrations of salicylates (Koop and Ghosh, 1994) followed by reports of effects on this and other intracellular signalling pathways and subsequent actions in controlling production of COX-2, iNOS etc with other NSAIDs (Paik et al., 2000; Allgayer, 2003; Bryant et al., 2003; Yoon et al., 2003; Rainsford, 2004) and naturally occurring anti-inflammatory agents (e.g. curcumin, ginger, resveratrol, various plant polyphenols) (Pellegratta et al., 2003; Grzanna et al., 2005; Yeh et al., 2003; Yoon & Baek, 2003; Bengmark, 2006) together with other studies on the cellular mechanisms of inflammation (Lo et al., 1998) have provided insight into the potential effects of regulating intracellular signalling as a means of controlling cytokines, COX-2, PLA2, iNOS and metalloproteinases and the actions of reactive oxygen species (ROS; oxyradicals) that are central to the inflammatory processes (Celec, 2004; Saklatvala, 2004; Jimi & Ghosh, 2005; Wu, 2005; Papa et al., 2006; see Figure 4). Thus, much effort has been devoted in recent years to discover and develop specific inhibitors of the various signalling pathways involved in inflammation (Saklatvala, 2004; Miwatashi et al., 2005; Bolos, 2005; Diller et al., 2005; Hynes and Leftheri, 2005; Goldstein and Gabriel, 2005; Kaminska, 2005; Peifer et al., 2006; Goldstein et al., 2006; Lin et al., 2006; Sabat...
Figure 4. Intracellular Signaling in Inflammation
Source: From Saklatvala (2004)

et al., 2006; Metzger et al., 2006; Kulkarni et al., 2006; Friedmann et al., 2006) many of which have strikingly different chemical structures (e.g. see Figure 5). While many of these agents are in early stages of development there are indications that some (e.g. see Saklatvala, 2004; Miwatashi et al., 2005) are orally active and effective in rheumatoid arthritis and some other chronic inflammatory diseases.

Figure 5. (Continued)
Source: (a) From Saklatvala (2004)
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Source: (b) From Friedman et al. (2006)

Figure 5. (Continued)

Source: (c) From Sabat et al. (2006)
Figure 5. Inhibitors of p38
Source: (e) From Kaminska (2005)
Other cell signalling systems that are thought to have potential as targets include the family of nuclear proteins known as Signal Transducers and Activators of Transcription (STAT), of which STAT1, which controls production of IFN-γ and growth hormone are of particular interest (de Prati et al., 2005). As with all signalling pathways which are so ubiquitous in cells, the specificity of attack by drugs on the components of these pathways will be a determinant of their utility. Any generalised inhibition of signalling pathways may have generic effects, some of which may be undesirable. Already, certain p38 MAP kinase inhibitors have been found to have broad actions that limit their safety in some cases and there has, as a consequence, been a move to develop drugs with inhibitory effects down-stream of post receptor pathways (Saklatvala, 2004).

6. NOVEL NON-ARTHRITIC USES OF NSAIDs

Over the past 3–4 decades, there have been a considerable number of developments involving the use of NSAIDs in conditions other than the treatment of arthritic disease and other painful states. These developments have mostly arisen from investigations of the pharmacological actions of these drugs or from chance pharmaco-epidemiological observations, not necessarily directly related to investigating the associations with NSAIDs. The first of these observations was by O’Brien (1968) who found that aspirin, but not salicylate, caused inhibition of the aggregation of platelets which was initially thought to be, and later confirmed as a factor in promoting bleeding from the gastroduodenal region (Rainsford, 2004b). It was not long before the “antiplatelet” effect of aspirin was exploited not only for its potential anti-inflammatory effects (Rainsford, 2004b) but also for prevention of thromboembolic- and coronary-vascular diseases, which is now legend (Webert & Kelton, 2004).

The initial observations that have lead to aspirin and other NSAIDs being recognized for their tumour-inhibiting and anti-metastatic properties have been somewhat less clear. It was known in the 1970’s that aspirin and some other NSAIDs could inhibit the growth and metastasis of tumour cells (Gasic et al., 1972; Wood & Hilgard, 1972; Powles et al., 1973; 1974; LiVolsi, 1973). There were also suggestions that oxyphenbutazone (Tanderil®) could have benefit in radiation treatment of otorhino-laryngeal tumours (JForl, 1976) by ameliorating inflammatory effects of radiation (Dargent, 1969; Klein et al., 1972; Muller-Fassbender et al., 1973). Multiple observations that there were marked increases in the concentrations of prostaglandins in tumours (Sandler et al., 1968; Bennett, 1971; 1976; Bennett & Del Tacca, 1975) as well as in cancer cell cultures (Jaffe et al., 1971; Levine et al., 1972) combined with prostaglandin effects on cell metabolism and proliferation (Makman, 1971; Van Wijk et al., 1972) and bone resorption and metastasis (Harris et al., 1973; Bennett et al., 1975; 1976) gave rise to the recognition that increased prostaglandin production was a major factor in the growth and proliferation of cancer. The recognition at about the same period (early 1970’s) that prostaglandin production could be inhibited by aspirin and other NSAIDs (Van, 1971; Ferriera
et al., 1971; Flower, 1974) and reduce the growth of tumours (see above) gave rise to the suggestion that aspirin and other NSAIDs could inhibit growth and proliferation of malignant tumours (Harris, 2003; Rainsford, 2004d; Rigas & Kashfi, 2005; Deans & Wigmore, 2005; Wang & Dubois, 2006).

Extensive investigations have now shown that NSAIDs have protective effects against colorectal cancer (Hull et al., 2003; Thun & Henley, 2003; Damjanovic et al., 2004; Sanborn & Blanke, 2005; Abir et al., 2005). NSAIDs have also been found to reduce the incidence of gastric tumours (Dai & Wang, 2006), two common brain tumours (i.e. gliomas and meningiomas) (Nathoo et al., 2004), breast cancer (Saji et al., 2004; Harris, 2004), cholangiocarcinoma of the biliary tract (Wu, 2005) and possibly adenocarcinomas and squamous cell carcinomas of the oesophagus or gastro-oesophageal junction (Tew et al., 2005). A whole range of other cancers are being considered for potential therapy with NSAIDs (Riedl et al., 2004; Claria & Romano, 2005; Kashfi & Rigas, 2005). Indeed the application of the NSAIDs, including those which have novel modes of action (e.g. inhibition of 5-lipoxygenase, production of nitric oxide from NO-NSAIDs) and what are regarded as non-COX-2 models of action are now broadening the focus of therapeutic attack in prevention and possibly even treatment of many pathological types of cancers (Rigas et al., 2003; Claria & Romano, 2005; Kashfi & Rigas, 2005).

NSAIDs have found particular application in the prevention of Alzheimer’s disease and other neurodegenerative conditions (Rogers, 2004; Piruzi and Practico, 2006). Their application arose from clinico-epidemiological observations of reduction in the risk of onset and development of Alzheimer’s patients taking NSAIDs (Rainsford, 1999a; 2004g; 2005d). Later investigations have found variable effects of different NSAIDs, but with some limited significant benefit from the coxibs or COX-2 selective agents (Rainsford, 2005a; Piruzi & Practio, 20–06) suggesting that there may be non-COX mechanisms important in the putative neuroprotection in this syndrome involving the actions of pro-inflammatory cytokines, oxyradicals and leucocyte activation (Rainsford, 2004g; 2005a).

Aspirin, ibuprofen and some other NSAIDs have been found to have benefit in preventing cataract, especially that attributed to diabetes mellitus, as well as in control of this and other perturbed metabolic states (Rainsford, 2004g).

7. FUTURE SCOPE

There is now enormous scope for the application of new and established anti-inflammatory agents with various receptors and targets for their actions as well as the development of novel anti-inflammatory drugs in the future. An immense array and variety of inflammatory reactions are now known to underlie serious chronic diseases and conditions which urgently require new therapeutic approaches centering on control of chronic inflammation. These include cardiovascular disease (Elhajj et al., 2004), transplantation reactions (Rocha et al., 2003), skin diseases including difficult conditions such as psoriasis (Lee et al., 2003; Skinner, 2004; Nash & Clegg, 2005), and actinic keratosis (Jorizzo, 2004), neurodegenerative
diseases including Alzheimer’s disease (Rogers and Lahiri, 2004), sepsis (Rice & Bernard, 2005), ageing [here aspirin has given encouraging results] (Phillips & Leeuwenburgh, 2004), and ophthalmic diseases (O’Brien, 2005). Each of these has a differing spectrum of inflammatory reactions and varying involvement of inflammatory mediators as well as cells of the immune system that regulate the inflammation. NSAIDs, each with differing mechanisms of action on inflammation, will continue to be exploited for therapy of these and other conditions and the design of drug delivery systems may help considerably where there is need to get focussed delivery of the drug at specific body sites e.g. skin, (Skinner, 2004) bone marrow etc. There are a large number of older NSAIDs and derivatives (e.g. see Rainsford, 1999, 2004) some of which have passed out of fashion, but these may have utility in some conditions where their mechanism of action suits the particular application. There are also the new signal transduction inhibitors that may have specific utility in a wide variety of inflammatory conditions by virtue of their mechanism of action. Anti-cytokine agents may eventually prove to be useful for chronic diseases not only by way of therapeutic benefits and targets for their actions, but also by way of showing “proof of principle” which can serve to encourage development of small molecules to control the action of target cytokines either at the level of cytokine receptors or post-receptor signalling events. Likewise, the successful application of nutraceuticals, a large number of which have been found effective in controlling inflammation (Shay and Banz, 2005), may ultimately lead to isolation of their active components (as shown with the grape component, resveratrol; Pellegatta et al., 2003) and the development of potent derivatives.

8. CONCLUSIONS

Many of the newer anti-inflammatory agents that have been developed since the turn of this century were discovered following the investigations of the mechanisms underlying the control of inflammatory conditions over the past 2–3 decades. We are now beginning to see an immense array of potential for these new drugs as well as the long-established drugs (NSAIDs, corticosteroids, DMARDs) and natural products (nutraceuticals). As better understanding of the mechanisms underlying chronic diseases progresses, so the applications of individual anti-inflammatory agents will be investigated and conditions for their optimal use and delivery established. There is a whole world of new opportunities awaiting the use of anti-inflammatory agents, both established and novel, in the future.

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