ANTIPYRETIC THERAPY

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1. ABSTRACT

Antipyresis can be achieved by physical methods such as cooling the body with tepid water or by pharmacological means such as the administration of antipyretic drugs. The nonsteroid anti-inflammatory drugs (NSAIDs) including aspirin, have been used to combat fever since the end of the 19th century and the analgesic antipyretics, from about the same time. Most of the antipyretic analgesics such as acetanilide and phenacetin are no longer used in therapy because of their toxicity. However, the metabolite of these two drugs, acetaminophen, became a highly popular antipyretic in the 1950s and is now the antipyretic of first choice in most developed countries.

The disadvantages of administering NSAIDs is their gastrotoxicity manifested as irritation, ulcers and bleeding of the stomach mucosa. Acetaminophen also is toxic to the liver in doses only slightly above the therapeutic dose. Thus, selective COX-2 inhibitors, which do not damage the stomach and are free fom hepatotoxicity, may be the drugs of choice for reducing fever in the future.

2. INTRODUCTION

Antipyresis can be achieved by physical means, i.e. to physically lower the temperature with external cooling or by pharmacological methods. Physical methods include immersion in cold water, blowing cold air from an electric fan over the patient or sponging with cool water, or an alcohol-water mixture. But these procedures alone are relatively ineffective because of compensatory thermogenesis (1). Frequently therefore, especially in children, antipyretic drugs such as acetaminophen are administered at the same time as the application of external cooling. More often though, the non-steroid anti-inflammatory drugs (NSAIDs) such as aspirin or antipyretic analgesics, for example acetaminophen, are used for antipyresis. In some countries, the selective cyclooxygenase-2 (COX-2) inhibitor nimesulide is administered (mostly for childhood fevers). Rofecoxib and etoricoxib, more selective on COX-2 than nimesulide, have not achieved widespread use as antipyretics although their use as analgesics in arthritis is well established (Table 1).

The review which follows describes the history, toxicity and mechanism of action of the commonly used antipyretic drugs and speculates on possible antipyretic agents of the future.

3. NON STEROID ANTI-INFLAMMATORY DRUGS (NSAIDS)

3.1. History of the NSAIDs

The treatment of fever, pain and inflammation goes back many thousands of years. It is mentioned in ancient writings from as early as Sumerian and ancient Egyptian times. These early treatments were based on the extraction and administration of salicylate derivatives from plant sources such as willow, spirea, meadowsweet or myrtle. Hippocrates recommended extracts of willow bark to relieve fever and the pain of childbirth around 400 BC (2).

Antipyretic Therapy

Table 1. Antipyretic drugs

Non-selective NSAIDs [1]	Antipyretic analgesics	Selective COX-2 Inhibitors
Aspirin	Paracetamol	Nimesulide
Indomethacin	Acetanilide	Meloxicam
Ibuprofen	Phenacetin	Etodolac
Piroxicam	Antipyrine	Celecoxib
Diclofenac	Amidopyrine	Rofecoxib
Naproxen	Dipyrone	Etoricoxib
Flurbiprofen		Valdecoxib
Fenoprofen		Lumiracoxib
Ketoprofen		
Fenbufen		
Tenoxicam		
Mefenamic acid		
Aceclofenac		
Sulindac		
Nabumetone		

[1] NSAIDs which inhibit both COX-1 and COX-2 non-selectively.

The first 'clinical trial' of willow bark was made by a country parson, the Reverend Edward Stone of Chipping Norton in Oxfordshire, England. In 1763, Edward Stone presented a report to the Royal Society on the use of willow bark in fever (3). He had tasted it and was surprised by its extraordinary bitterness, which reminded him of the taste of cinchona bark (containing quinine), then being used to treat malaria. He believed in the 'doctrine of signatures' which dictated that the cures for diseases would be found in the same locations where the malady occurs. Since the "willow delights in a moist and wet soil, where agues chiefly abound" , he gathered a pound of willow bark, dried it over a baker's oven for 3 months then ground it to a powder. His greatest success was with doses of 1 dram (equivalent to 1.8 grams), which he reported using in 50 patients with safety and success. He concluded his paper by saying, "I have no other motives for publishing this valuable specific, than that it may have a fair and full trial in all its variety of circumstances and situations, and that the world may reap the benefits accruing from it".

The active substance of the common white willow, a glycoside of salicylic acid known as salicin, was isolated in 1829 by Leroux, who also demonstrated its antipyretic properties. The Italian chemist, Piria, converted salicin to salicylalcohol and thence to salicylic acid. Salicin was used in 1876 in an important study by TJ MacLagan, a Dundee physician (4). He took 2g of salicin and since he experienced no adverse effects himself, gave it to patients with rheumatic fever. The result of the treatment was a reduction of body temperature, pain and inflammation, thus demonstrating the antipyretic, analgesic and anti-inflammatory effects for which salicylates were subsequently used. By 1874, other physicians began to use synthetic salicylic acid, which was being produced commercially by a method formulated by Kolbe and his colleague, Lautemann, in Germany.

In the 1880s sodium salicylate replaced salicylic acid for the treatment of chronic rheumatoid arthritis, because of its greater solubility and lower toxicity. The success of salicylate encouraged the pharmaceutical company of Frederick Bayer to search for a derivative of salicylic acid with comparable or superior efficacy. The head of the chemical research laboratory at Bayer, assigned this project to a young chemist named Felix Hoffman. Hoffman also had personal reasons for finding a more acceptable derivative of salicylic acid. His father had been taking salicylate for many years to treat his arthritis and found he could no longer tolerate the unpleasant taste of the drug. In the scientific literature, Hoffman found a reference to the synthesis of acetylsalicylic acid by the French chemist, Charles Gerhardt in 1853. Acetylsalicylic acid was then synthesised by Felix Hoffman in 1899, tested in Bayer's pharmacology laboratories (5) and found to be palatable and effective by Hoffman's father. The new drug was named "Aspirin" by Bayer's chief pharmacologist, Heinrich Dreser; a name probably derived from *Spiraea,* the genus of plants to which meadowsweet belongs. Meadowsweet was a popular source of salicylic acid before the organic synthesis became established and the acid extracted from it would have been "Spirsaüre". Acetylation of Spirsaüre produced "Acetylspirsaüre" which was soon shortened to Aspirin.

Soon, clinical studies were being carried out on aspirin such as those of Kurt Witthauer and Julius Wohlgemuth in 1899 (6,7), which quickly demonstrated that aspirin was a much better analgesic for relieving headaches than salicylic acid. By the early 1900s, the main therapeutic actions of aspirin were established as antipyretic, anti-inflammatory and analgesic effects. In the 1960s, other aspirin–like drugs were discovered such as mefenamic acid, ibuprofen and indomethacin and in the early 1970s, naproxen, tolmetin, ketoprofen and sulindac became approved as anti-inflammatory agents. They were all effective in lowering yeast-induced fever in rats (8) and demonstrated anti-inflammatory effects tested by carrageenan-induced oedema in the same species (9). Although the drugs in this group are chemically diverse, most of them are organic acids and share to a large extent the same therapeutic properties. In varying doses they alleviate the swelling, redness and pain of inflammation, reduce fevers of diverse aetiologies and cure a headache. They also share to a greater or lesser extent a number of similar side effects. Depending on dose, they can cause gastric upset, in high doses delay the birth process and in overdose may damage the kidney. A particularly interesting "side effect", now known as a therapeutic action, is the anti-thrombotic effect. These shared actions of the aspirin-like drugs prompted pharmacologists to search for a common mode of action for the therapeutic actions and side effects of this group of substances.

Meanwhile, during the 1930s, several reports appeared of a group of versatile local hormones found in seminal fluid that contracted uterine smooth muscle and also caused a fall in blood pressure. Von Euler identified the active principle of seminal plasma as a lipid-soluble acid, which he named "prostaglandin" because he thought it originated from the prostate gland (10). Technical advances in the 1960s allowed the characterisation of the

prostaglandins (PGs) as a family of lipid compounds with a 20-carbon unsaturated carboxylic acid structure and a cyclopentane ring (11). In 1964, Bergström and co-workers (12) synthesised PGE_2 using arachidonic acid and an enzyme preparation from ram seminal vesicles, thus demonstrating these organs as the true sources of prostaglandins in the semen.

Many biochemical theories were suggested to explain the mechanism of action of aspirin-like drugs including uncoupling of oxidative phosphorylation and inhibition of dehydrogenase enzymes. But, in 1971, at the Royal College of Surgeons in England, John Vane and his colleagues (13) discovered that aspirin and similar drugs inhibit the enzyme that generates prostaglandins.

As synthetic prostaglandins became available, Milton and Wendlandt (14, 15) studied the effect of a number of prostaglandins on deep body temperature in conscious cats. They initially found that PGE_1, injected into the cerebroventricular system of the conscious cat produced a marked rise in body temperature. This febrile response to PGE_1 was not affected by the antipyretic drug, acetaminophen which had previously suppressed fever produced by the central administration of endotoxin (16). In 1971, Milton and Wendlandt (15) showed that PGE_1 and PGE_2 were equally potent in their hyperthermic effects in the conscious cat, and that PGE_1 was hyperthermic in the rabbit and rat. These observations were confirmed by Feldberg and Saxena in 1971, who also located the site of action of PGE_1 as the pre-optic area of the anterior hypothalamus (17).

Milton and Wendlandt showed in 1970 (14), that a prostaglandin-like substance was released into the cerebrospinal fluid during pyrogen-induced fever in the cat. Feldberg and Gupta in 1973 (18) removed cerebrospinal fluid from the third cerebral ventricle of the conscious cat and assayed it for contractile prostaglandin activity on the rat fundus strip preparation of Vane (19). The prostaglandin activity was very low in afebrile animals, but increased during fever produced by injecting bacterial pyrogen directly into the third cerebral ventricle. Administration of acetaminophen reduced both the fever and the contractile activity of the cerebrospinal fluid. Feldberg *et al.* in 1973 (20), also identified the prostaglandin released into the cerebrospinal fluid of the conscious cat during endotoxin-induced fever as PGE_2 by thin-layer chromatography of the cerebrospinal fluid, followed by bioassay and radioimmunoassay. Aspirin, acetaminophen and indomethacin all abolished fever and at the same time lowered the prostaglandin-like activity of the cerebrospinal fluid.

Prostaglandin-like activity was also measured in human cerebrospinal fluid obtained from pyrexic patients with bacterial or viral infections (21). PGE-like activity was found in the cerebrospinal fluid of patients suffering from viral encephalitis or pyrogenic meningitis and no activity was present in the cerebrospinal fluid of afebrile patients.

Vane therefore proposed, in 1971 (13), that, since fever could be mimicked by the release of prostaglandins and because of the proposed involvement of prostaglandins in inflammation and pain, the prostaglandins were mediators in all three of these pathological conditions and the non-steroidal anti-inflammatory drugs produced their antipyretic, anti-inflammatory and analgesic actions by inhibiting the synthesis of prostaglandins. This proposal provided an answer to the question of why aspirin-like drugs should have these apparently dissimilar therapeutic actions. The work of Vane and his colleagues published in 1971 (13, 22, 23) confirmed that aspirin, indomethacin and salicylate reduced the synthesis of prostaglandins by inhibiting the enzyme which produced them, and thus manifested their antipyretic, anti-inflammatory and analgesic effects.

3.2. Toxicity of NSAIDs

Gastrointestinal side effects of the NSAIDs can vary from mild dyspepsia to severe upper gastrointestinal complications manifested as bleeding and perforations resulting in hospitalisation. From the ARAMIS (Arthritis, Rheumatism and Aging Medical Information System) data banks it has been estimated that in the US about 107,000 hospitalisations occur each year associated with NSAID use and 16,500 deaths (24). The so called 'cytoprotective' action of prostaglandins in preventing gastric erosions and ulceration is mainly brought about by endogenously produced prostacyclin (PGI_2) and PGE_2, which reduce gastric acid secretion and exert a direct vasodilator action on the vessels of the gastric mucosa. In addition to these major actions, prostanoids stimulate the secretion of viscous mucus, gastric fluid and duodenal bicarbonate. Removal of the cytoprotection provided by prostaglandins with NSAIDs thus results in the typical gastrointestinal toxic effects of this group of drugs (25, 26).

Another important side effect of the NSAIDs is their anti-thrombotic effect through inhibition of the synthesis of thromboxane A_2 in platelets which prevents blood clotting. Aspirin is particularly effective in preventing clotting of blood since it inhibits prostaglandin synthase (cyclooxygenase; COX) irreversibly by acetylation of the enzyme (Figure 1). This is the basis for administration of daily low-dose aspirin to prevent heart attacks (27).

Prostaglandins do not maintain renal blood flow in normal, healthy kidneys, but prostaglandin production becomes important in maintaining blood flow of compromised kidneys, for example in disease states such as congestive heart failure, liver cirrhosis or renal insufficiency (28). Inhibition of prostaglandin synthesis results in the renal side effects of NSAIDs which include reduced renal blood flow and glomerular filtration rate, sodium retention and hyperkalaemia (29). Severe renal side effects occur in approximately 1% of patients taking NSAIDs, and 3% of all cases of renal failure are reported to be caused by NSAIDs (30).

3.2. Mechanism of action of NSAIDs

A homogeneous, enzymatically active cyclooxygenase (COX) or endoperoxide synthase (PGHS) was isolated in 1976 (31). This membrane-bound haemo-
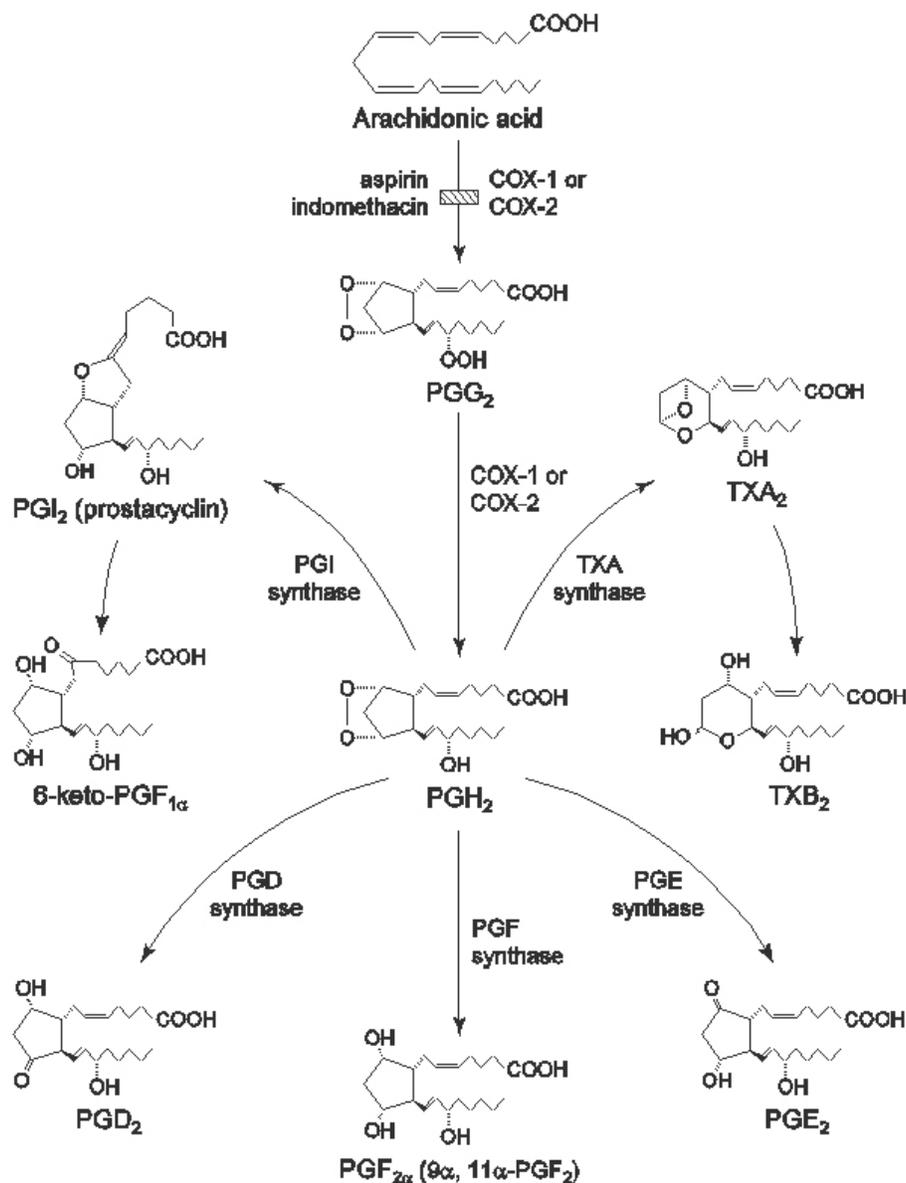
Figure 1. The Arachidonic Acid Cascade. Arachidonic acid is converted by cyclooxygenase-1 (COX-1) or cyclooxygenase-2 (COX-2), located on the endoplasmic reticulum of most cells, to unstable endoperoxides (PGG_2 and PGH_2). COX-1 is constitutive and produces prostaglandins (PGs) essential for maintaining homeostatic mechanisms and COX-2 becomes induced by cytokines, mitogens and inflammatory mediators to release PGs involved in pathological states such as inflammation. Cytosolic prostaglandin synthases which vary in different cells then metabolize PGH_2 to different prostanoid products. Thus, vascular endothelial cells and gastric epithelial cells make primarily prostacyclin (PGI_2) which protects the endothelium from platelet thrombi and stomach mucosa from damage by gastric acid. Blood platelets synthesize thromboxane A_2 which promotes the formation of thrombi and clotting of blood. PGD_2 is a mediator produced mainly in the brain and in mast cells. $PGF_{2\alpha}$ is synthesised in reproductive organs such as the uterus and ovary, and is involved in pregnancy and parturition. PGE_2 has an important role centrally and peripherally controlling temperature and mediating pain. It is also produced by cells of the immune system involved in inflammation and is released from many cells such as those in the gastrointestinal tract, lungs, and kidneys to maintain homeostatic functions.

and glycoprotein with a molecular weight of 71 kDa is found in the endoplasmic reticulum of all cells in the body except the red blood cells. It exhibits COX activity which both cyclises arachidonic acid and adds the 15-hydroperoxy group to form PGG_2. The hydroperoxy group of PGG_2 is reduced to the hydroxy group of PGH_2 by a peroxidase that utilizes a wide variety of compounds to provide the requisite pair of electrons. Both COX and hydroperoxidase activities are contained in the same dimeric protein molecule (Figure 1).

The COX active site is a long hydrophobic channel, with tyrosine 385 and serine 530 situated at the apex of the channel. Aspirin irreversibly inhibits COX by acetylation of the serine 530, thereby excluding access for arachidonic acid, whereas other NSAIDs, such as flurbiprofen, inhibit COX by competing with arachidonic acid for binding to the active site. In 1991, Xie *et al* published the discovery of a second COX gene which expressed a COX isoenzyme now named COX-2 (32). Aspirin and other NSAIDs were non-selective inhibitors of both COX-1 and COX-2. COX-1 is the constitutive so called 'housekeeping enzyme', synthesising prostaglandins which protect the stomach mucosa from damage, aggregate platelets when required and maintain kidney function. COX-2 can be induced by inflammatory stimuli such as bacterial lipopolysaccharide and the induction prevented by glucocorticoids. Inhibition of COX-2 accounts for the anti-inflammatory actions of the NSAIDs and inhibition of COX-1 is responsible for the unwanted side effects (33) such as damage to the upper gastro-intestinal tract mucosa.

Although the protective prostaglandins of the upper gastro-intestinal tract are normally synthesised by COX-1, in the absence of COX-1, an upregulated COX-2 can take over the synthesis (34). Thus, COX-1 gene-deleted mice do not develop gastrointestinal damage unless they are also treated with a selective COX-2 inhibitor (35; 36) and simultaneous inhibition of both COX-1 and COX-2 is required to produce lesions of the gastric (37; 38) or upper intestinal mucosa (34; 36). Similarly, COX-2 may not be the sole enzyme involved in the inflammatory response. Prostaglandins synthesised by COX-1 may also take part in mediating inflammation such as carrageenin-induced pleurisy in rats. Gilroy *et al* (39) reported that NSAIDs selective for inhibition of COX-1, such as piroxicam or aspirin, produce a longer-lasting inhibition of inflammation than selective inhibitors of COX-2.

The amino acid sequence of COX-2 cDNA shows a 60% homology with the sequence of the constitutive enzyme but the mRNA for the inducible enzyme is approximately 4.5 kb while that of COX-1 is 2.8 kb. Both enzymes have a molecular weight of 71 kDa and similar active sites for the natural substrate and for blockade by NSAIDs, except that the active site of COX-2 is slightly larger than that of COX-1 and also has an additional side pocket (40).

Evidence from recent studies implicates inhibition of COX-2 as a major mechanism of the antipyretic action of the NSAIDs. Li *et al* (41) found that COX-2 knockout mice did not develop fever with bacterial lipopolysaccharide whereas COX-1 gene-deleted mice showed a normal pyretic reaction to lipopolysaccharide injection. Moreover, patients with fever due to various bacterial and viral infections responded to therapy with a selective COX-2 inhibitor by a reduction of their fever (42). It has been postulated that COX-2 in endothelial cells lining small blood vessels in the hypothalamus is upregulated by circulating lipopolysaccharide and that this enzyme synthesizes PGE_2 responsible for fever (43). The endothelial cells are situated outside the blood brain barrier and thus the COX-2 is accessible to inhibition by circulating NSAIDs. The activity of the endothelial cells may be modulated by mediators released from perivascular cells which also synthesize PGE_2 through induction of COX-2 and could contribute to the fever response (44). A recent report suggests that the development of fever involves a more complex mechanism in which COX-1 is also involved (45) and the immediate fever which follows an intravenous injection of lipopolysaccharide into guinea pigs is unlikely to be mediated by PGE_2 synthesised by inducible COX-2 (46). The authors suggest (46) that this fever is mediated by a constitutive form of COX-2.

Febrigenic PGE_2 may also be synthesised in response to lipopolysaccharide by peripheral structures (47; 48) as well as by endothelial cells in the hypothalamus. PGE_2 from peripheral organs is most likely to cause the first phase of biphasic fever in rabbits, while the second phase is probably mediated by endotoxin acting in the hypothalamus (47). Peripheral PGE_2 generated in the vicinity of sensory vagal afferents would act as the stimulus for initiation of fever (49; 50).

4. ANTIPYRETIC ANALGESICS

4.1. History of antipyretic analgesics
The development of antipyretic analgesic drugs in the late nineteenth century as by-products of the German dye industry, marked a milestone in antipyretic therapy. Physicians of the time were keen to bring down fever in their patients and used various methods, such as bleeding, cupping etc to achieve this. Acetanilide was the first of this group to be synthesised and it is considered to be the parent compound of the antipyretic analgesics. It was made by Gerhardt in 1853 by reacting aniline with acetylchloride or acetic anhydride. Its therapeutic action was discovered accidentally in 1886 by Cann and Hepp (51), two physicians working in Strasburg, who were mistakenly supplied with acetanilide by their pharmacist. They had ordered a supply of naphthalene to treat intestinal worms but were instead provided with a substance which had no effect on the worms but very effectively reduced the temperature of febrile patients. This drug was then named "antifebrin" and was subsequently found to be analgesic as well as antipyretic.

However, acetanilide proved to be too toxic and a number of para-nitrophenol derivatives were then tested, among them phenacetin, also known as acetophenetidin. Hinsberg and Kast (52) introduced phenacetin into therapy in 1887. Morse had already synthesised acetaminophen in 1878 (53) by the reduction of para-nitrophenol to para-aminophenol followed by acetylation of the product. The first clinical trial of acetaminophen was carried out by von Mehring in 1893 (54), who declared that in spite of its antipyretic and antineuralgic properties, acetaminophen had side effects which precluded its therapeutic use. However, the following year, Hinsberg and Treupel (55) showed that 500mg of acetaminophen was safe and as effective as 700mg phenacetin or 1g antipyrine in reducing fever, and phenacetin, acetanilide and antipyrine became established as useful antipyretic analgesics. It was some years before

acetaminophen achieved popularity in spite of being known as a major metabolite of both acetanilide and phenacetin (56, 57).

Acetaminophen became popular in the 1940s, when it was rediscovered as the most important metabolite of acetanilide and phenacetin in humans (58, 59). It was first marketed in the USA in 1950 and subsequently in the UK in 1956. Several studies in the 1950s established its efficacy in reducing fever (60) and relieving pain caused by cancer (61), dental surgery (62) or arthritis (63). Between 1957 and 2000, almost 100 clinical studies of the antipyretic action of acetaminophen, mostly in young children with various infections, many of the upper respiratory tract (64) were published. Although some of the trials were designed to examine the efficacy of acetaminophen in preventing febrile convulsions in very young children, this action could not be conclusively established from the outcome of the tests. A comparison of the antipyretic effect of acetaminophen with that of a similar dose of aspirin in 30 volunteers treated with endotoxin, found that acetaminophen was more effective in reducing the endotoxin-induced fever (65). Exceptionally, for example in febrile children (66) or HIV-infected patients (67), paracetamol produces an unexpected hypothermic response when the body temperature can fall as low as 35°C.

4.2. Toxicity of antipyretic analgesics

Of the para-aminophenol derivatives only acetaminophen is still in use today. The use of acetanilide was abandoned early on due to the marked methemoglobinemia, cyanosis and functional anemia that it produced. Phenacetin is also no longer available due to its association with methemoglobinemia and haemolytic anemia when chronically overused. Even though 75%-80% of administered phenacetin is converted to acetaminophen by the liver, acetaminophen itself causes less methaemoglobinemia and haemolytic anemia than phenacetin. Both drugs, however, can produce liver necrosis and acetaminophen may cause hepatotoxicity after a single dose of 10-15g. It is therefore not recommended that adults should take more than a total of 2.6g daily and children no more than 1.2g a day (68).

Acetaminophen hepatotoxicity has been explained through its conversion by the Cytochrome P450 2E1 isoenzyme pathway to a highly reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI). This metabolite binds to cell proteins and DNA resulting in acute hepatotoxicity and becomes detoxified by being conjugated with glutathione. Thus, when liver glutathione stores are low, as in chronic alcoholism or starvation, the risk of liver toxicity with acetaminophen becomes greater (69, 70). The antidote to poisoning with acetaminophen is N-acetylcysteine, which replenishes the reduced glutathione stores in the liver.

The pyrazolone derivatives which include antipyrine, aminopyrine and dipyrone are no longer sold in the USA or in most of Europe. Aminopyrine and antipyrine were taken out of therapeutic use because of their potentially fatal bone-marrow toxicity and administration of aminopyrine or its congener, dipyrone, frequently caused irreversible agranulocytosis due to the formation in the plasma of antibodies to leukocytes (71).

Chronic abuse of any of the antipyretic analgesics can result in analgesic nephropathy in sensitive individuals. The primary kidney lesion may be characterised by papillary necrosis, followed by chronic interstitial nephritis (68).

4.3. Mechanism of action of antipyretic analgesics

It is generally accepted that the antipyretic analgesics are unlikely at therapeutic doses to produce stomach irritation, bleeding or perforations, the characteristic side effects of the aspirin-like drugs. In addition, unlike the NSAIDs, these drugs have no effect on platelet aggregation or increase in bleeding time in patients (68, 72). They also have no, or only weak, anti-inflammatory actions. Thus, it has been suggested that acetaminophen is a weak inhibitor of peripheral COX-1 and COX-2 and that most of its therapeutic activity is directed at the central nervous system (73, 74, 75). This is borne out by the fact that antipyretic analgesics cross the blood brain barrier without difficulty and become distributed homogeneously throughout the organism (76), unlike the acidic NSAIDs which accumulate locally at inflammatory sites (77).

There is support for this concept in recent studies by Chandrasekharan and his co-workers (78), who have characterised a novel variant of COX-1 present mostly in the brain. The authors have designated this enzyme as COX-3, but some sources suggest that COX-1b would be a more appropriate classification. This variant of COX-1 localised mainly in the central nervous system of the dog has a unique sensitivity to inhibition by the drugs classified as antipyretic analgesics, namely acetaminophen, antipyrine, aminopyrine, dipyrone and phenacetin. The COX-3 mRNA in human tissues has also been investigated and may be involved in synthesising PGE_2, which promotes fever and pain and which can be selectively inhibited by acetaminophen and related drugs. It is hoped that development of new, selective inhibitors of COX-3 will provide potent antipyretic and analgesic drugs without the liver toxicity of acetaminophen.

5. SELECTIVE COX-2 INHIBITORS

5.1. Nimesulide

Nimesulide was developed by Riker Laboratories in the early 1970s and granted a patent in Belgium and the USA in 1974 (79). It has been sold over the counter in Italy since 1985 and subsequently in many other European and South American countries. From the beginning it was regarded as a rather unusual drug. Unlike other NSAIDs, it was a weak inhibitor of the cyclooxygenase from sheep seminal vesicles but effectively reduced yeast-induced hyperthermia in rats and carrageenan-induced oedema of the rat's hind paw (80, 81). However, at anti-inflammatory doses which reduced carrageenan-induced paw edema, nimesulide had no effect on gastric prostaglandin levels and did not cause bleeding of the rat gastric mucosa (82, 83).

Using purified enzymes *in vitro*, nimesulide was shown to be 5 times more selective towards COX-2 than COX-1 (84, 85) and in the human whole blood assay it was 20 times more potent in inhibiting COX-2 in monocytes than COX-1 in platelets (86). A study in 20 patients demonstrated that therapeutic doses of nimesulide had no effect on urinary excretion of thromboxane metabolites in contrast to therapy with aspirin which reduced urinary thromboxane metabolites by more than 50%. Both nimesulide and aspirin in therapeutic doses reduced excretion of prostacyclin metabolites, due to inhibition of endothelial COX-2 by these drugs (85).

In rats, following administration of brewer's yeast an oral dose of nimesulide was more active than acetaminophen in reducing body temperature (87), while it was equally as active and safe as acetaminophen in elderly pyrexic patients with upper or lower respiratory tract infections (88). In children with viral or bacterial infections of the respiratory tract, nimesulide produced a more rapid reduction of fever than other NSAIDs or acetaminophen (89, 90, 91).

5.2. Meloxicam
When measured by the human whole blood assay, meloxicam has a selectivity for COX-2 of approximately 10 times that for COX-1 (92). Its antipyretic action has been demonstrated in yeast-induced fever in rats (93) and on fever produced with endotoxin in cats (94). It has been registered for therapeutic use in osteoarthritis and rheumatoid arthritis in Europe and the USA.

5.3. Celecoxib, valdecoxib
There is no published information available on the antipyretic action of celecoxib (95) or valdecoxib, both marketed by Pfizer. Both are selective for COX-2; by the human whole blood assay celecoxib is 10 times more selective and valdecoxib 30 times more selective in inhibiting COX-2 than COX-1. Thus, they are very likely to have antipyretic activity in fever in humans.

5.4. Rofecoxib, etoricoxib
Rofecoxib was introduced into medical practice by Merck Frosst in 1999 (96). It easily enters the central nervous system and has potent antipyretic, analgesic and anti-inflammatory activity. In the human whole blood assay, the ratio of IC50 against COX-1 in platelets to IC50 against COX-2 in monocytes is 38, which indicates a strong selectivity for COX-2 versus COX-1 (97). Rofecoxib dose-dependently reversed endotoxin-induced pyresis in rats with an ID50 of 0.24mg/kg and in squirrel monkeys with an oral dose of 3mg/kg (42). In a double blind randomised placebo-controlled trial in 94 patients with fevers from 38oC to 40oC due to upper respiratory tract or systemic viral infections, 25mg rofecoxib decreased the oral temperature to baseline levels within 4 hours after administration (42).

Merck's successor to rofecoxib, etoricoxib with a selectivity for COX-2 of 106, also lowered endotoxin-induced fever in squirrel monkeys by 81% with a dose of 3mg/kg (98).

5.5. Toxicity of selective COX-2 inhibitors
Until recently, nimesulide was considered to be no more toxic than other NSAIDs and to have a less damaging effect on the gastic mucosa than the acidic aspirin-like drugs. However, in March 2002 nimesulide was withdrawn from the Finnish market because of unacceptable hepatotoxicity and then from the Spanish market in May 2002. Data from the Spanish Pharmacovigilance System reported a higher rate of hepatic injury with nimesulide than with other NSAIDs in Spain during the 1990s (99).

In large scale clinical trials, meloxicam was less toxic to the stomach mucosa than diclofenac or piroxicam (100). However, post marketing surveillance reports indicate as great a toxicity for the stomach as non-selective NSAIDs. This most likely reflects that meloxicam is prescribed primarily for those patients who are at greatest risk of developing gastric damage (101).

In a six month randomised, controlled trial (95) celecoxib was shown to be less damaging to the stomach mucosa than ibuprofen or diclofenac at doses greater than clinical doses. However, when aspirin was taken simultaneously for prevention of heart attacks, the advantage of selective COX-2 inhibition was lost. Also, when the trial was continued for 12 months no difference was evident in gastric damage caused by celecoxib or the control drugs.

Rofecoxib has been shown to be much less toxic to the stomach mucosa than the non-selective NSAIDs (96). However, in the multicentre VIGOR trial a higher incidence of myocardial infarction was recorded in the group of patients receiving rofecoxib than was found in the comparator group treated with naproxen. This could be explained by a specific toxic action of rofecoxib or a cardioprotective effect of naproxen, similar to the anti-platelet effect of low dose aspirin. The marketing of rofecoxib's successor, etoricoxib, has been delayed in the USA until this question has been resolved, although etoricoxib is already available for osteoarthritis and rheumatoid arthritis in Europe.

5.6. Mechanism of action of selective COX-2 inhibitors
The marketed selective COX-2 inhibitors have sulphonanilide or sulphonamide rather than acidic structures and are freely distributed throughout the system including into the brain. The active site of COX-2 is very similar to that of COX-1 except that it is slightly larger and has a side pocket which accommodates the more bulky molecules of the selective COX-2 inhibitors. These are too large to fit the active site of COX-1 and thus do not effectively inhibit prostaglandin synthesis in the stomach mucosa (102). The antipyretic action of the selective COX-2 inhibitors is mediated by inhibition of the COX-2 enzyme located close to the *organum vasculosum laminae terminalis* (OVLT) area of the hypothalamus, most likely in the endothelial cells lining the hypothalamic blood vessels thus preventing synthesis of hyperthermic PGE_2 (43).

6. FUTURE PERSPECTIVES

Because of the toxic actions of the NSAIDs, acetaminophen and nimesulide, the selective COX-2 inhibitors such as rofecoxib or etoricoxib may achieve widespread use in the future as antipyretic agents of choice.

Alternately, potent inhibitors of COX-3 may be developed without the toxicity of acetaminophen which will replace acetaminophen as drugs of choice for antipyresis.

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