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Antinociception and the New COX Inhibitors: Research Approaches and Clinical Perspectives

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ABSTRACT

New generations of cyclooxygenase (COX) inhibitors are more potent and efficacious than their traditional parent compounds. They are also safer than the classic non-steroidal anti-inflammatory drugs (NSAIDs) and are starting to be used not only for low to moderate intensity pain, but also for high intensity pain. Three different strategies have been followed to improve the pharmacological profile of COX inhibitors:

1. Development of COX-2 selective inhibitors. This is based on the initial hypothesis that considered COX-2 as the enzyme responsible for the generation of prostaglandins only in inflammation, and, therefore, uniquely responsible for inflammation, pain and fever. Initial expectations gave rise to controversial results, still under discussion. The second generation of these compounds is being developed and should contribute to clarifying both their efficacy and the specific functions of the COX enzymes.

2. Modified non-selective COX inhibitors. Molecules like nitro-NSAIDs or tromethamine salt derivatives have been synthesized considering that both COX-1 and COX-2 are responsible for the synthesis of prostaglandins involved either in homeostatic functions or inflammation. Nitroaspirin, nitroparacetamol or dexketoprofen trometamol are some examples of molecules that are already showing an important clinical efficacy. The modifications performed in their structures seem to lower the unwanted side effects as well as to enhance their analgesic efficacy.

3. Combined therapy of classic NSAIDs with other drugs. This strategy looks for improvements in the incidence of adverse effects or to take advantage of the synergistic enhancement of their therapeutic effects. Some of the molecules resulting from these strat-

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egies are very valuable as therapeutic agents and open a wide range of possibilities in the treatment of high intensity pain, including neuropathic pain, and opiate sparing therapy.

INTRODUCTION

Low to moderate intensity pain has been traditionally treated with NSAIDs, a type of drugs considered for decades as the first step in the treatment of pain, and especially pain resulting from inflammatory processes. Although NSAIDs are members of a very large and chemically diverse family of compounds, they share a common mechanism of action. As first described by Vane in 1971 (128) they are inhibitors of COX enzymes and therefore, of the synthesis of prostaglandins. Prostaglandins are involved in the generation of pain, fever and inflammation, but they are also involved in many other physiological processes and systems, such as reproductive processes, cardiovascular and renal systems, gastrointestinal (GI) and respiratory tracts, etc., whose inhibition by NSAIDs leads to a well-known number of side effects.

COX inhibitors of new generation are safer, and are more efficacious than their traditional parent compounds, they have been even compared with opiates (91). In addition, they are starting to be used not only in low to moderate intensity pain but also in high intensity pain. Thus, the large number of prescriptions written every year is not surprising. An estimated 70 million prescriptions are written and 30 billion over-the counter medications are sold annually in the USA (62) More than 21 million prescriptions are written in England, at a cost of more than 180 million pounds per year (74). It is also not surprising that the number of reports on the activity of NSAIDs published in 2001 was well over 3900. This certainly reveals an enormous interest in these compounds.

The published studies revealed a lack of agreement on the mechanism of action of these drugs. In many cases, the intensity of the effects observed after administration of new COX inhibitors is greater than expected from the blockade of prostaglandin synthesis only. Some experiments performed with modified molecules show effects that were not evident with the parent compounds. These findings suggest new mechanisms of action, that are possibly independent of COX inhibition. There is also a controversy regarding mechanisms of adverse effects of these compounds. One of the theoretical advantages of the new COX inhibitors is that they might induce fewer side effects and would be well tolerated by patients suffering from gastric or renal pathology, or in chronic therapy. Their adverse effects are, however, so different from the older drugs that sometimes it is not possible to determine whether a new NSAID is more useful than the better-known parent molecule. This controversy is probably impossible to avoid since different molecules may have different effects, depending on the pathological situation, individual tolerability, dosage and protocol of treatment as well as other factors that are difficult to control.

It is important to clarify whether the new molecules under study or recently marketed have more advantages or disadvantages in the treatment of pain than the classical NSAIDs. In this manuscript we reviewed research approaches to the development of new NSAIDs, the efficacy of the new families of COX inhibitors, focusing on their antinociceptive activity, their mechanisms of action, the benefits in using them and the future prospects for these compounds.

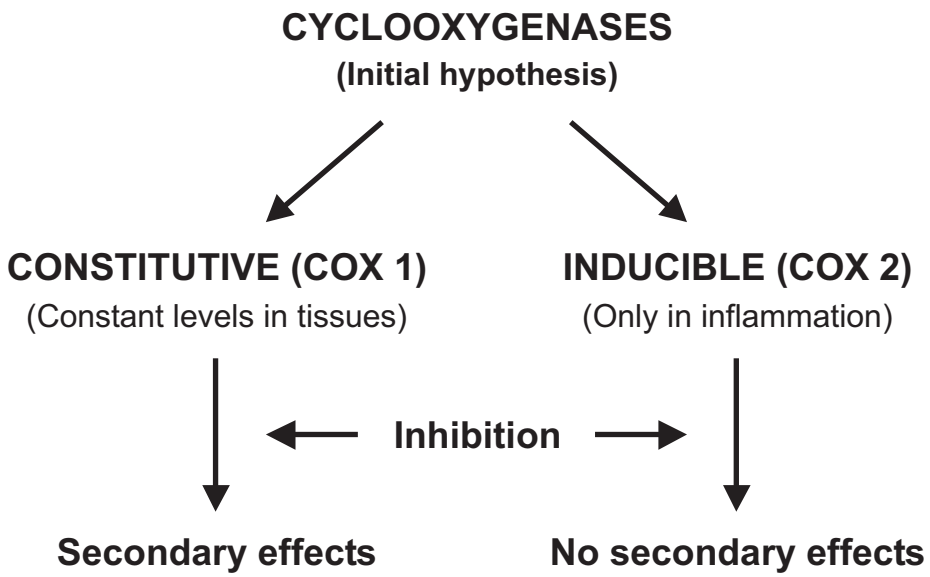


Fig. 1. COX-1 has been considered as the "house-keeping" enzyme, responsible for the synthesis of prostaglandins involved in several homeostatic processes and inflammation, pain and fever. The blockade of COX-1 by classic NSAIDs would induce a degree of anti-inflammation and reduce pain and fever, as well as several side effects, due to the inhibition of the physiological actions of prostaglandins. COX-2 was initially thought to be responsible for the synthesis of prostaglandins only in the presence of inflammation. Its blockade by selective COX-2 inhibitors would, therefore, induce analgesia, anti-inflammatory effect and reduce fever without adverse effects.

RESEARCH APPROACHES TO THE DEVELOPMENT OF NEW COX INHIBITORS

One of the starting points for the development of new NSAIDs was the discovery of the COX-2 or inducible COX (103,147), encoded by a different gene from the constitutive COX form or COX-1. The inhibition of COX-1 not only causes reduction of pain, fever and inflammation, but also induces unwanted side effects due to the blockade of physiological functions of the enzyme. COX-2 was initially thought to be absent from most healthy tissues. Its expression was thought to be rapidly induced in inflammation by cytokines and bacterial products, like lipopolysaccharides (70,104). Therefore, COX-2 was expected to be involved in the synthesis of prostaglandins in inflammation only, whereas COX-1 would be responsible for the synthesis of prostaglandins in most homeostatic functions. The discovery of COX-2 led to the development of selective enzyme inhibitors that might have better or similar clinical efficacy than classic NSAIDs but fewer adverse effects, since they would not block the synthesis of prostaglandins in homeostatic functions (Figs. 1 and 2). The first COX-2 selective inhibitors, the coxib family, were marketed in 1999 and are a major success of the pharmaceutical industry. General practitioners, as well as patients suffering from pain, considered these types of drugs the new era analgesics because of the theoretical safety of these compounds as well as their efficacy as anti-inflammatory and analgesic drugs.

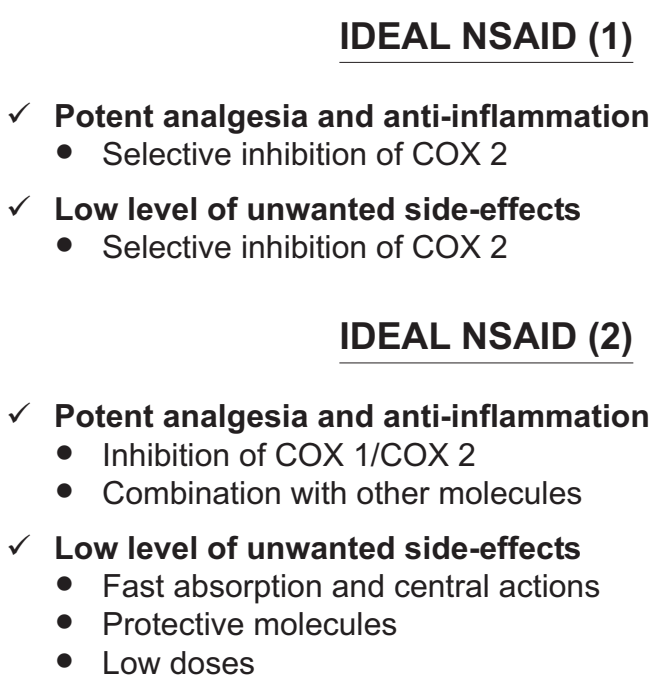


Fig. 2. Two main approaches to the development of new COX inhibitors are based on the specific role of each of the COX isoenzymes in inflammation. 1. The ideal NSAID would be capable of selectively inhibiting the COX-2 enzyme. This would cause a potent anti-inflammatory effect and analgesia, but would lack unwanted side effects, since COX-2 would not be involved in homeostatic functions. 2. All prostaglandins are involved in the generation and/or maintenance of pain and inflammation. Inhibition of both, COX-1 and COX-2, is needed to obtain a potent therapeutic effect. The adverse effects could be diminished by enhancing the potency of a COX inhibitor. The required dose can be reduced or by an increment in the GI absorption and central effects. Also, a combination with protective molecules would help in the reduction of the adverse effects.

A second approach to the development of COX inhibitors considered that any prostaglandin, synthesized by the action of the COX isoenzymes, was involved in the generation of inflammation, pain and fever, and, therefore, the inhibition of both COX isoenzymes was necessary in order to produce an efficient pain relief (Fig. 2). Conversely, the sole inhibition of COX-2, would prevent patients from having cardiovascular protection, the only secondary effect resulting from the inhibition of COX-1 beneficial for many patients. New non-selective COX inhibitors have been developed based on these arguments.

The unwanted side effects, resulting from the inhibition of the homeostatic functions achieved by prostaglandins, have been reduced by improving the potency and/or the absorption of the drugs, and, therefore, reducing the dose needed, or by enhancing the central actions of some classic NSAIDs (129). This strategy is growing in acceptance by authors who consider that a dual inhibition of COX-1 and COX-2 enzymes, with improved gastric tolerance, is preferable to a selective COX-2 inhibition (57). This approach has been supported by the discovery that COX-1 was overexpressed in inflammation, and that COX-2 was also present under physiological conditions in some tissues (65,144). This implies that the inhibition of both isoenzymes should be necessary to achieve a

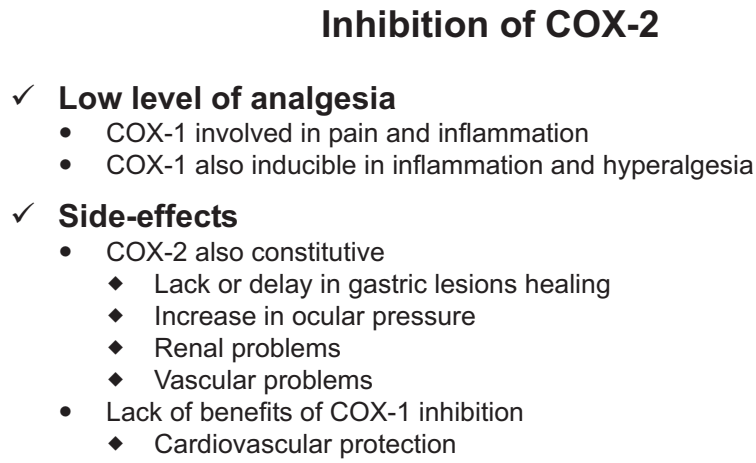


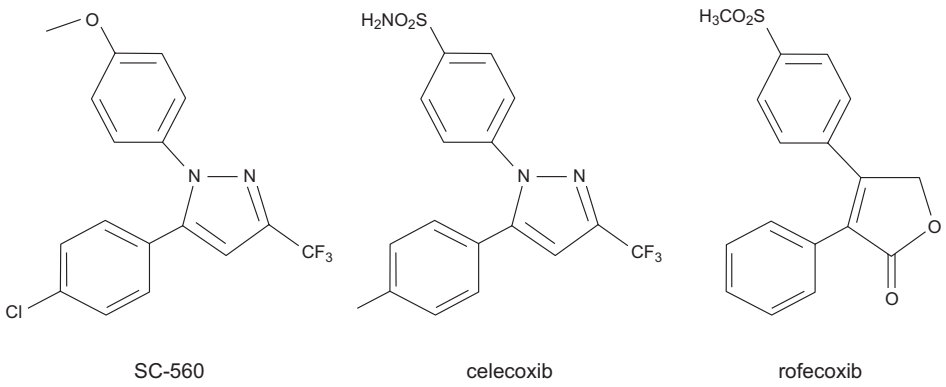
Fig. 3. The first generation of COX-2 selective inhibitors has shown a lower analgesic potency than expected, as well as the appearance of unwanted side effects. In contrast, the selective inhibition of COX-2 lacks the platelet aggregation inhibitory effect. Since COX-1 seems to be involved also in pain and inflammation and COX-2 seems also to be constitutive in some tissues, some authors have proposed a combined inhibition of COX-1 and COX-2 in order to obtain a potent therapeutic effect and cardiovascular protection.

strong pain reduction and, conversely, that the inhibition of either of the isoenzymes would induce some degree of unwanted side-effects (Fig. 3; 92,132,133).

Finally, a third strategy in the development of new NSAID-derived therapy was combination therapy of COX inhibitors with other compounds. In some cases, this therapy was based on the search for a protection from the adverse effects caused by the classic NSAIDs. In other cases the rationale for this therapy was based on the synergistic, or at least supra-additive, enhancement of the analgesic and anti-inflammatory effects observed by the combined administration of COX inhibitors with other types of drugs involved in the processing of nociceptive information or in the generation and maintenance of the inflammatory responses.

COX-2 SELECTIVE INHIBITORS

Since the role of COX-2 was supposed to be only related to inflammation or/and sensitization due to inflammatory processes, the prostaglandins derived from its action would not be implicated in homeostatic processes and, therefore, their inhibition would block the generation of pain, fever and inflammation, but not homeostatic functions (Fig. 1). These arguments led to the development of selective enzyme inhibitors that might have similar or even better clinical efficacy than classic NSAIDs but fewer adverse effects. The coxib compounds have been the first family of selective COX-2 inhibitors. This family of compounds included celecoxib (105) and rofecoxib (32), as the first generation of compounds (Fig. 1), and valdecoxib, parecoxib, etoricoxib and others, not yet on the market, as the second generation. These molecules are diarylheterocyclic derivatives and their COX-2 selectivity is due to the insertion of a sulfonamide or sulfone group in their structure (65; Fig. 4). Therefore, the molecules are much larger and cannot fit into the COX-1 binding



SC-560 celecoxib rofecoxib

Fig. 4. SC-560 is a COX-1 selective inhibitor that belongs to the diarylheterocycle family of drugs. These types of compounds become very selective COX-2 inhibitors when a sulfonamide or sulfone group is inserted in their structure. Examples of these molecules are the first generation of COX-2 selective inhibitors celecoxib and rofecoxib.

site, but they can bind into the larger site of the COX-2 structure. Celecoxib, for instance, has a structure very similar to that of SC-560 (122), a very selective COX-1 inhibitor, the difference being almost exclusively in the insertion of a sulfonamide group (Fig. 1). As a result, celecoxib and rofecoxib are very selective COX-2 inhibitors, 200 to 800 times more selective for COX-2 than for COX-1, depending on the preparation or assay used.

Clinical studies

Following the initial enthusiasm and logical expectations with these compounds, contradictory observations have been made on the analgesic efficacy and secondary effects caused by selective COX-2 inhibitors. The observations range from no real advantage over conventional, non-selective NSAIDs, in the treatment of dental pain (74) or arthritis (71), to a clear advantage in the therapeutic use of these drugs in similar conditions (28,52,83). Malmstrom et al (86), in a double-blind study carried out on 272 patients with postoperative dental pain, observed an important analgesic efficacy of rofecoxib that was superior to that of celecoxib. The analgesic efficacy of rofecoxib was comparable to that of ibuprofen, but the duration of the effect was longer (>24 h vs. 8.9 h) and the safety profile was similar across all treatment groups. The results observed in this study were similar to those of previous studies showing an analgesic efficacy of rofecoxib indistinguishable from that observed with ibuprofen (42). Coxibs were effective in the treatment of osteoarthritis in several studies (see12 for review). The analgesic effect of celecoxib, for example, was studied in 3, 248 patients with osteoarthritis in phase III trials, showing a good tolerability, with an analgesic effect comparable to that of naproxen (13). In another randomized double-blind controlled trial carried out on 784 adults with osteoarthritis of the knee or hip, rofecoxib was well tolerated and its efficacy was clinically comparable to that of diclofenac (28). In patients with rheumatoid arthritis, rofecoxib was effective and better tolerated than naproxen (121). Also, celecoxib was effective in rheumatoid arthritis, with an analgesic and anti-inflammatory activity similar to that of diclofenac but with a lower incidence of GI adverse events (44). Other studies, however, concluded that there was no real advantage in the use of rofecoxib and celecoxib over classic NSAIDs. Celeco-

xib, for example, showed a lower efficacy than naproxen in a phase III trial carried out on patients with osteoarthritis (12). According to Jeske (74), rofecoxib and celecoxib do not seem to be more effective than conventional NSAIDs (e.g., ibuprofen) in the management of dental pain and inflammation.

Important discrepancies are also found in the prevalence of adverse effects in patients treated with COX-2 selective inhibitors. In a randomized double-blind study performed on healthy adults, for instance, renal side effects were insignificant in patients treated with the COX-2 selective inhibitor MK966, whereas indomethacin significantly decreased glomerular filtration rate (29). Contradictory results (19) have been observed in clinical studies involving a large number of patients. These studies show that the overall incidence of renal adverse events after treatment with celecoxib was greater than that after placebo, but similar to that after NSAIDs (141). In healthy elderly subjects, COX-2-specific inhibition may spare renal hemodynamic function, although the effects on sodium excretion, as well as on the urinary excretion of prostaglandin E_2 and 6-keto-prostaglandin $F_{1\alpha}$, appear to be similar to those of nonspecific COX inhibitors such as naproxen (142). Other studies show that COX-2 selective inhibitors were effective in the treatment of rheumatoid arthritis and osteoarthritis, but side effects, such as headache, diarrhea, abdominal discomfort and dizziness, have been reported with celecoxib (60); similar side effects have been observed with other non-selective COX inhibitors. Also, some authors expressed caution and concerns with the use of COX-2 selective inhibitors (19,72,87), since suppression of COX-2 can result in exacerbation of inflammation-associated colonic injury (109), gastric perforation (195), elevation of blood pressure (101) and induction of leukocyte adherence (101). Some authors (63) concluded that, at comparable doses, COX-2 inhibitors and conventional NSAIDs have similar effects on the renal function. It was surprising, however, that mice with a disruption of the gene encoding COX-2 showed normal inflammatory responses but severe nephropathy (99), indicating that COX-2 might be necessary for the normal function of the renal system.

With respect to gastrointestinal (GI) safety, some clinical trials have shown a better tolerability of celecoxib when compared to naproxen (13) or rofecoxib compared to diclofenac (28) or naproxen (121) or to ibuprofen (78). Two recent and large controlled clinical trials (see 17 for more details) have analyzed the GI safety of coxibs: the Vioxx Outcomes Research (VIGOR) trial (16) and the Celecoxib Long-term Arthritis Safety Study (CLASS; 120). The VIGOR study showed that rofecoxib was associated with significantly fewer clinically important upper GI events than naproxen. The CLASS study showed similar advantages of celecoxib over diclofenac or ibuprofen, though, in this case, the differences in the incidence of ulcer complications were not statistically significant (see ref. 17 for further discussion). Other studies, however, have shown an important incidence of serious side effects in patients treated with either rofecoxib or celecoxib, leading to the conclusion that there is no real advantage in the use of selective COX-2 inhibitors over classic NSAIDs (107). Also, COX-2 does not seem to be involved in platelet aggregation, and, therefore, COX-2 selective inhibitors are not useful in cardiovascular protection; they may even be potentially harmful in patients at risk for thrombosis (30,51,100).

The second generation of coxib inhibitors is not yet on the market, but they seem to be more efficacious than the first generation of coxib compounds. Valdecoxib, for instance, seems to be more efficacious then rofecoxib in the relieving of pain associated with oral surgery (53). Clinical studies are also starting to show that valdecoxib might be safer and

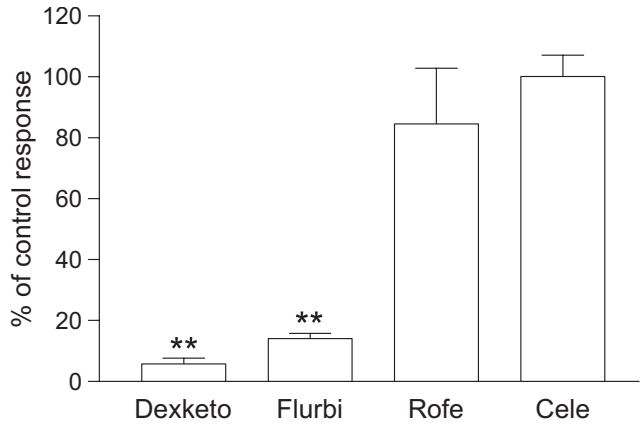


Fig. 5. The non-selective inhibition of COX enzymes by dexketoprofen trometamol (Dexketo) and flurbiprofen trometamol (Flurbi), by acute systemic administration, resulted in a potent antinociception in monoarthritic rat spinal cord reflexes. In similar experiments, however, the administration of the COX-2 selective inhibitors rofecoxib (Rofe) and celecoxib (Cele) did not cause a significant reduction of the nociceptive responses. The reason for this lack of effect might be related to an acute protocol of administration, but it is also possible that an inhibition of both COX enzymes is needed to observe a fast and effective antinociception (data taken from ref. 92).

more effective than other COX inhibitors in the relief of pain after minor surgery (35). Its injectable prodrug, parecoxib sodium, has shown a better GI tolerability than either ketorolac or naproxen (64).

Animal studies

Controversial results have been observed in animal models of nociception. The selective COX-2 inhibitor SC-58125, for instance, was effective in the prevention of edema and hyperalgesia when injected 1 h prior to the induction of inflammation by carrageenan (118). In this case, the dose used was very high and, according to some authors, the effect was probably due to a non-specific inhibition of COX enzymes (137). Chang et al. (32) observed that rofecoxib inhibited the carrageenan-induced edema and reversed the hyperalgesia with a potency similar to that observed with indomethacin. Similar results were observed with celecoxib (105,122) and DFU (110). In withdrawal reflexes recorded in animals with carrageenan-induced inflammation, no significant antinociceptive activity was observed after the acute administration of celecoxib or rofecoxib in either soft-tissue inflammation or arthritis induced by the administration of carrageenan (92). However, the administration of the non-selective COX inhibitors dexketoprofen trometamol or flurbiprofen trometamol induced a potent and effective reduction of responses to noxious stimuli (Fig. 5).

In the chronic inflammation model, murine chronic granulomatous tissue air pouch, aspirin was more effective than the COX-2 preferential inhibitors nimesulide and NS-398 at inhibiting granuloma dry weight, vascularity and COX activity (57). Also, the study of the antinociceptive and anti-inflammatory effect of different COX-2 preferential inhibitors in animals with carrageenan-induced inflammation, led to the conclusion that effective actions were observed only at doses capable of inhibiting COX-1. At these doses, a signi-

ficant suppression of gastric prostaglandin synthesis and gastric mucosal erosions were also observed (137).

The inconsistencies among these results might be related to the different timing of the administration of the drugs, relative to the stage of the inflammation. COX-2 selective inhibitors are effective against carrageenan-induced pleuresy when tested 3 h after the administration of carrageenan (32,122), but they were without effect 6 h later, a time at which non-selective COX inhibitors still showed efficacy (58). This interesting observation might explain the contradictory results observed with NSAIDs, and has led to the hypothesis of the existence of a third type of COX involved in nociception and inflammation (145); a hypothesis recently confirmed (31). It is also possible that, despite the evidence shown by early experiments, COX-2 is not involved as much as COX-1 in the development or maintenance of inflammation. Knockout mice lacking the gene responsible for the expression of COX-2 showed similar inflammation to that observed in wild-type animals (41,99,137). However, mice lacking the gene responsible for COX-1 showed a significant reduction of the inflammatory responses (79).

The important divergence in the efficacy and potency of COX-2 selective inhibitors observed in both clinical and animal studies might, therefore, be related to diverse causes. An importance of COX enzymes and prostaglandins in the generation and/or maintenance of inflammation, may depend on many other factors. These include: the timing of the inflammatory reaction, the cause of inflammation, the previous condition of a patient, a possible existence of other COX enzymes, the interaction or modulation of COX activity by other systems or even the protocol for administration of COX inhibitors. Some of the unwanted secondary effects observed in clinical trials may be due to the loss of selectivity of these compounds after their administration at huge doses (9). The widespread popularity of coxibs and the popular belief that they are completely safe might have led to prescription (or even self-administration by patients) of these drugs at very high doses at which the drugs might interact with COX-1 enzyme or other systems. Further studies with the second generation of coxib compounds may clarify the situation.

NEW NON-SELECTIVE COX INHIBITORS

Tromethamine Derivatives of NSAIDs-Isomers

The experimental evidence suggests that the blockade of prostaglandins synthesized in peripheral tissues does not fully explain the analgesic actions of the NSAIDs (93). Effects at sites located in the central nervous system have been reported in several experimental preparations (see for review 129), and might be responsible in part for the analgesic action of COX inhibitors. The structure of a NSAID is critical for its rate of absorption and penetration into the central nervous system. Although most NSAIDs cross the blood brain barrier to varying degrees, the search for NSAIDs with a preferential central site of action and an easy penetration into the central nervous system is an important challenge since they are more likely to induce a potent analgesic action, especially in situations of hyperalgesia due to central sensitization. Ketoprofen and flurbiprofen are well-studied examples of effective non-selective COX inhibitors with an important, if not predominant, action at a central site or sites. These NSAIDs, like other 2-arylpropionic acids, cross the blood-brain barrier in humans rapidly and induce potent antinociceptive actions in several mo-

dels of pain. The effect of these compounds on the inhibition of prostaglandins is stereoselective, due to the dextrorotatory enantiomer: (+)-(S)-ketoprofen, or dexketoprofen and (+)-(S)-flurbiprofen (21). Although the R-enantiomers do not seem to cause GI toxicity themselves, they seem to participate in its generation since the ulcerogenic effect is greater after the administration of racemic mixtures than after the treatment with the S-enantiomers (139). The S-enantiomers of flurbiprofen and dexketoprofen have, therefore, been marketed in an attempt to benefit from the potent analgesic actions of these compounds, as well as the lower incidence of their adverse effects, compared to the racemic mixtures. Also these compounds inhibit platelet aggregation and, therefore, retain cardiovascular protection.

Furthermore, tromethamine salts of dexketoprofen and S-flurbiprofen have all the properties of free acids, but are more rapidly absorbed by the gastric mucosa. The time needed to achieve their maximum plasma concentration is shorter and gastric ulceration is less likely (7,27,89). In plasma, however, the tromethamine salt is rapidly hydrolyzed and ketoprofen or flurbiprofen recover their lipophilicity and their ability to enter the central nervous system. Therefore, in cerebrospinal fluid (CSF) their concentration is higher than that of the parent compounds. The therapeutic dose and the level of unwanted side effects are, therefore, lower (89). A similar rationale has been followed in the release of other COX inhibitors with a tromethamine salt in their molecule, as for instance ketorolac tromethamine (115). The success of these compounds made them to the oral therapy of choice in humans.

Clinical studies

Clinical studies with tromethamine-salt derivatives have been very encouraging. Dexketoprofen trometamol is rapidly absorbed after oral administration in healthy volunteers, with a maximum plasma concentration time between 0.25 and 0.74 h, whereas for the free acid (7,8) this time was between 0.5 and 3 h. It also showed very good tolerability and greater analgesic potency than the racemic compound (7,8). In other studies, dexketoprofen trometamol was also well tolerated, showing a good efficacy in postoperative dental pain, and a higher analgesic effect than that observed with dipyrone (4) and a more rapid onset of action than the racemic ketoprofen (94). In migraine patients, the intensity of pain significantly decreased 30 min after the oral administration of a single dose of 25 mg of dexketoprofen trometamol. Also, the incidence of accompanying symptoms (nausea, vomiting, photophobia, and phonophobia) significantly decreased after the treatment, with no serious adverse symptoms reported during the study (1). Dexketoprofen trometamol 25 mg was also more effective and better tolerated in the treatment of osteoarthritis than ketoprofen 50 mg (10). It was also effective in reducing significantly the degree of morning stiffness in patients with osteoarthritis at an oral dose of 50 mg (114).

Animal studies

Dexketoprofen trometamol is a very effective depressor of spinal cord nociceptive reflexes. In normal anesthetized rats, the antinociceptive potency of dexketoprofen trometamol was comparable to that of μ-opiates, not only on responses evoked by natural stimulation but also on those evoked by electrical stimulation, including wind-up (69,91, see also below). The antinociceptive effect of dexketoprofen trometamol in anesthetized rats was significantly greater than that observed in similar experiments with the racemic

mixture of ketoprofen (68). In non-anesthetized mice, dexketoprofen trometamol was also a very effective antinociceptive agent after oral administration, and more potent than NSAIDs, like diclofenac (26). S-flurbiprofen is a potent anti-inflammatory and antinociceptive drug in carrageenan-induced inflammation (25), and the analgesic potency of ketorolac tromethamine has been compared to that of opiates in major surgery (148). Either dexketoprofen-trometamol or S-flurbiprofen-trometamol, were very effective by acute systemic administration in reducing spinal cord nociceptive reflexes in animals with soft-tissue inflammation and in animals with arthritis (92). In similar experiments, however, neither rofecoxib nor celecoxib showed any significant effect. This observation might imply a need to inhibit both COX isoenzymes in order to induce potent and fast analgesia, at least in acute preparations (92, see Fig. 5). In conclusion, the S-enantiomers of 2-arylpropionic acids combined with tromethamine salt share all the benefits of non-selective COX inhibitors with a fast absorption and fewer side effects than their parent compounds, and are a good option in the oral treatment of moderate to high intensity pain.

Nitro-NSAIDs

Nitric oxide (NO) is an important modulator of diverse physiological functions, such as regulation of blood flow and vascular tone in many tissues, platelet aggregation, activity of mastocytes as well as inflammation. It is also a neurotransmitter and neuromodulator in both the CNS and the periphery (88). NO also participates in GI activity; it is involved in the maintenance of mucosal blood flow, modulates the repair and integrity of the GI mucosa, and exhibits gastroprotective properties against different types of agents. Nonetheless, high concentrations of NO are related to pathological processes, including peptic ulcer or chronic inflammatory bowel diseases (88). Low doses of NO have, therefore, similar actions to those observed with prostaglandins, and this led to the hypothesis that a combination of COX inhibitors with NO donor molecules might be beneficial. NO-releasing derivatives of non-steroidal anti-inflammatory drugs (NO-NSAIDs) were, therefore, developed to take advantage of the cytoprotective property of NO (20,143). This effect was expected to compensate for the unwanted side effects of drugs, like aspirin or other NSAIDs (39), and would counterbalance the undesirable effects of prostaglandin inhibition (126).

Three molecules form nitro-derivatives: the parent NSAID molecule, the NO donor and a spacer that allows the synthesis of different derivatives (75). For instance, two different nitro-derivatives of aspirin have been produced (NO-ASA, Fig. 6), NCX4215, a 2-acetoxybenzoate 2-(2-nitroxy)-butyl ester, and NCX4016, a 2-acetoxy-benzoate 2-(2-nitroxy-methyl)-phenyl ester (39,75). Other examples are the diclofenac derivatives: nitrofenac or 2-[(2,6-dichlorophenyl)amino]benzeneacetic acid 4-(nitrooxy)butyl ester (11,22) and nitrosothiol esters of diclofenac (6).

Clinical studies

NO-derivatives of COX-inhibitors have shown an important therapeutic potential as anti-inflammatory agents, with a lower incidence of side effects than their parent compounds, and an enhanced gastric safety profile (22). Though most data come from animal experiments, there are already a few encouraging clinical studies that support similar benefits of NO-NSAIDs in humans. In healthy volunteers, for instance, the treatment with nitroaspirin for one week did not induce macroscopic changes in the GI mucosa, whereas

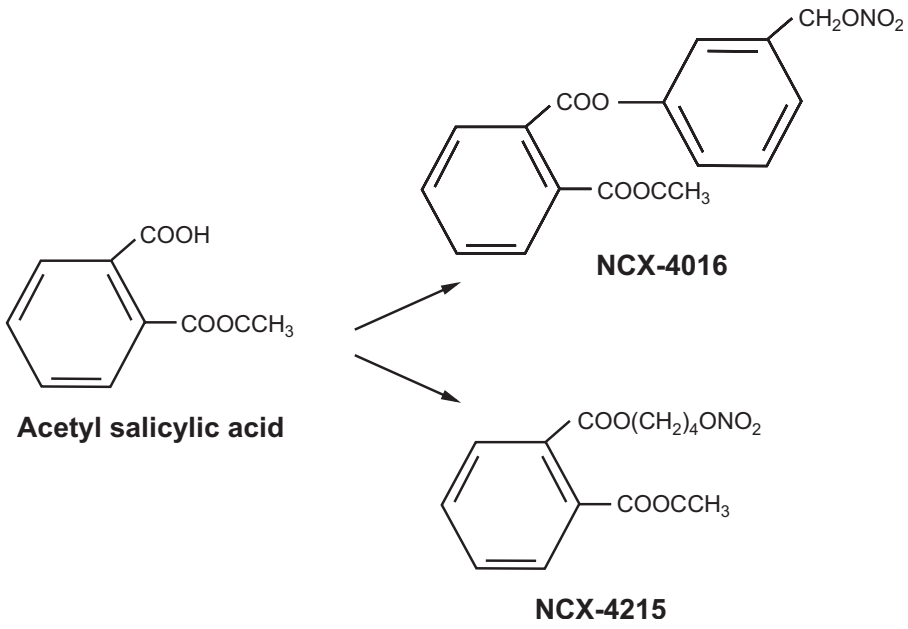


Fig. 6. Molecular structure of acetylsalicylic acid and two of its nitro derivatives. The combined action of COX-inhibitors and NO donors has been shown to be more effective in the reduction of inflammation and in antinociception than the effect observed by the sole inhibition of COX enzymes.

this toxicity was common in the majority of volunteers taking aspirin (116). Also, in contrast to aspirin, subjects treated with nitroaspirin showed no difference in GI toxicity in comparison with placebo and exhibited a wider anti-platelet profile than aspirin (50). Surprisingly, NO derivatives are, in most studies, more potent than their parent compounds. Nitroaspirin, for example, shows more potent antithrombotic activity than acetylsalicylic acid (98) and is more effective in *in vitro* antiplatelet tests (82).

Animal studies

Treatment of rats with aspirin or nitroaspirin inhibited carrageenan-induced hyperalgesia and acetic acid-induced abdominal constrictions. The antinociceptive potency achieved by nitroaspirin was greater than that of aspirin, with ED_{50}s of around two fold lower than those needed with aspirin to observe the same effect (3). The antinociceptive effects of aspirin and nitroaspirin were, however, similar in the tail withdrawal assay and in formalin-induced behavior (3).

NO-diclofenac produced analgesic and anti-inflammatory activity similar to those observed with diclofenac, but GI toxicity was not observed (6). NO-flurbiprofen and NO-ketoprofen significantly attenuated the increase in paw volume induced by the intraplantar injection of carrageenan, and, as in the previous cases, GI toxicity was lower than with the parent compound (134). Nitronaproxen caused superior antinociceptive effects to naproxen in the acetic acid-induced writhing model (37). In reducing edema formation nitronaproxen had activity either similar to that of the parent compound (33), or it had a much

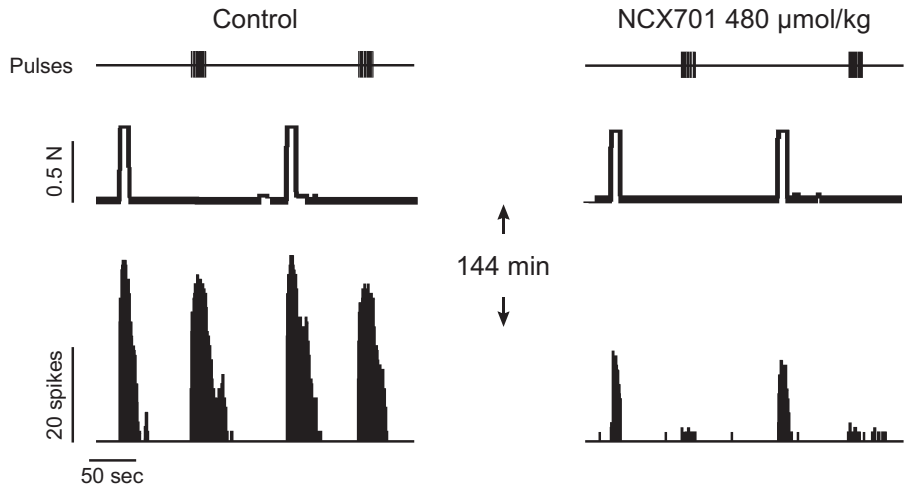


Fig. 7. The figure shows the activity recorded in a single motor unit evoked by electrical stimulation and noxious mechanical stimulation before and after the administration of nitroparacetamol or NCX701. The nitro-derivative of paracetamol, was an effective compound in the reduction of rat nociceptive responses to either noxious mechanical stimulation or high intensity electrical stimulation (wind-up). In these experiments, however, the acute systemic administration of paracetamol was not effective (data taken from ref. 111).

longer effect than that observed with naproxen (34). In one of these studies (33), the anti-nociceptive effect induced by nitronaproxen was significantly more pronounced than that observed with naproxen. Both compounds modulated T cell proliferative response in arthritis. However, nitronaproxen significantly reduced tumor necrosis factor alpha (TNF_{α}) and interleukin 1β (IL-1β) plasma levels, whereas naproxen reduced IL-1β but not TNF_{α} levels. A better GI tolerability was observed in rats with soft-tissue inflammation or arthritis after treatment with nitronaproxen compared to naproxen (34).

NCX-701, or nitroparacetamol, the nitroderivative of paracetamol (acetaminophen) is, by i.p. administration, an effective antinociceptive and anti-inflammatory agent in soft-tissue inflammation (2), despite the fact that paracetamol is devoid of anti-inflammatory activity (59,117, see also below). Also, paracetamol causes acute liver toxicity, whereas NCX-701 seems to be much safer, due to a hepatoprotective activity exhibited by NO (49,54). In our laboratory (Fig. 7), we have observed that NCX-701 is effective, by i.v. administration, as an antinociceptive agent in single motor unit activity evoked by either noxious mechanical stimulation or high intensity repetitive electrical stimulation (wind-up) in the normal non-inflammatory situation (111, Fig. 7). In this situation, however, paracetamol did not show any significant activity.

We also observed in monoarthritic animals that paracetamol and NCX-701 are potent and effective antinociceptive drugs in spinal nociceptive reflexes activated by noxious mechanical stimulation. The effect and potency were similar with the two drugs, although NCX-701 showed more time dependency than paracetamol (112). By oral administration to conscious rats, NCX-701 seems to be also very effective. In this case, and in contrast to that observed after i.v. administration, an important reduction of the level of hyperalgesia was observed at 30 min after administration of the drug. This time period was too short for

paracetamol to cause a significant reduction of the nociceptive response (112). A faster oral absorption due to the local action of NO, and, therefore, an increase in the amount of drug absorbed, might be the cause of more rapid onset of NCX-701 action compared to that of paracetamol by oral administration. Nevertheless, a COX-independent mechanism of action, or a combination of an independent action with an improved absorption, would also explain higher potency of NCX-701. In fact, in the latter experiments, NO-paracetamol was effective in reducing inflammation induced in soft-tissue by the intraplantar administration of carrageenan, an observation made also in other experiments (2). This effect supports a mechanism of action of NO-paracetamol that is different from that of paracetamol, since paracetamol has been previously shown to be an effective analgesic drug that is devoid of the anti-inflammatory action (59,117). It is not yet possible to precisely define the mechanism of this anti-inflammatory effect, but it has been suggested that some of the anti-inflammatory properties of NO-NSAIDs may represent the consequence of a reduction in the inflammatory response due to inhibition of generation of T helper 1 (Th1)-type cytokines or their actions. This mechanism is likely to involve inhibition of caspase proteases (46).

Other possibilities should also to be considered. Nitroparacetamol, for instance, was very effective as an antinociceptive drug either in responses to noxious mechanical stimulation or to high intensity electrical stimulation (wind-up). An action in the central nervous system is supported by the strong effect exerted on the wind-up phenomenon. In this phenomenon, repetitive electrical stimulation induces a progressive increase of nociceptive responses from spinal cord neurons (69, and references within) and is mediated by N-methyl D aspartate (NMDA), (36,40) and neurokinin-1 (NK1) receptors (38). A reduction of wind-up implies an inhibitory action of the circuitry involved in its generation, which is located in the central nervous system at the spinal cord level (69), although a modulation of the system at higher levels in the CNS is also possible (66). Therefore, the effect of nitroparacetamol takes place presumably at central sites rather than in the periphery, and although the actual mechanism of action remains unclear, it might influence the excitatory actions of the NMDA/NK1 systems.

It is also possible that the progressive release of NO favored or promoted an enhancement of the antinociceptive effect of paracetamol by a different mechanism of action. Although we have not seen any clear effect after the administration of the NO-donor NOC-18, NO donors may induce either pronociception or antinociception, and the clearest effect in spinal cord-mediated withdrawal reflexes seems to be antinociceptive (125). These actions appear to be NO concentration-dependent (77,125). Also, NO synthesis in the CNS is mediated by the constitutive neuronal isoform of the NO synthase, which seems to be positively modulated by the activation of NMDA (76). It has been suggested that NO release, because of NMDA receptor activation, modulates negatively the release of glutamate by enhancing the amount of monoamines in the synaptic space (76). Alternatively, prostaglandin E_2 (PGE_2) enhances the release of glutamate and aspartate in the spinal cord (102) and the blockade of COX activity might reduce the levels of PGE_2 and, therefore, the release of glutamate. If this is true, nitroparacetamol might negatively modulate the activity of NMDA receptors by a combined action of the NO released and the paracetamol molecule, depressing the release of glutamate through different mechanisms and causing an effect greater than that observed when administered separately. This would explain the potent effect of nitroparacetamol observed in the wind-up phenomenon (111,112). Further experiments are needed to assess this possibility.

As mentioned above, the incidence of adverse effects is lower with nitroderivatives than with their parent NSAIDs. In rabbit hearts subjected to low flow ischemia-reperfusion, nitroaspirin caused a more pronounced protection than aspirin (113). In rats, the oral administration of aspirin resulted in a time-dependent and dose-dependent gastric injury that was associated with apoptosis and caspase upregulation. Z-VAD.FMK, a pancaspase inhibitor, and NO donors protected gastric mucosa from acute damage induced by aspirin. Nitroaspirin spared the gastric mucosa and inactivated caspase by S-nitrosylation. Inhibition of TNF_{α} release or activity by TAPI-2 or anti-TNF_{α} receptor monoclonal antibodies protected against mucosal damage and caspase activation. Nitroaspirin protected gastric chief cells from toxicity induced by TNF_{α} by activating cGMP-dependent pathways (47). Wallace et al. (138) found that aspirin, at 30 mg/kg or higher doses, caused extensive gastric damage in rats. However, there was no detectable damage after the administration of equimolar doses of nitroaspirin. Nitroaspirin even seems to be protective to GI mucosa in cases of hemorrhagic shock (39,135,136) or injury induced by HCl and ethanol (126). The acute administration of NO-flurbiprofen or NO-ketoprofen in rats induced lower gastric toxicity than their parent compounds, and a daily administration of NO-flurbiprofen during one week did not cause any mucosal bleeding as did flurbiprofen (134). A GI protection after oral administration of other nitro-derivatives has been observed, but only NO-NSAIDs inhibited COX activity in GI mucosa (37). For instance, by oral administration to rats, nitrofenac, a diclofenac nitro-derivative, produced a significant acceleration of gastric ulcer healing (43). This implies that the GI protective effect was independent of the COX inhibition, and was probably due to the NO donor moiety. NO compounds seem, therefore, to be capable of protecting the GI mucosa from injury, possibly through a preservation of the mucosal blood flow (22).

A protection against experimentally-induced liver damage has also been observed in animals treated with nitroaspirin (48). Liver toxicity is a severe side effect that sometimes occurs after administration of paracetamol. This effect was not observed with nitroparacetamol (54). It is also important to note that NO-NSAIDs do not cause a significant decrease in arterial blood pressure, even when administered intravenously (111,112). The reason might be that the release of NO by these compounds is very slow. Lower toxicity or protection in other tissues or systems have also been observed with other nitro derivatives (22).

It can be concluded that this family of compounds are very promising drugs for the treatment of many different diseases that are accompanied by pain and inflammation. These compounds might represent an important therapeutic choice as oral analgesic drugs.

COMBINED THERAPY OF NSAIDs AND OTHER MOLECULES

The observation that the combined administration of some COX inhibitors with other drugs induces an enhancement or even synergy of some of their therapeutic actions (85) led to the development of combined prescriptions in the treatment of pain of severe intensity. The combination of NSAIDs with other drugs has been used either to enhance the analgesic and anti-inflammatory properties of these drugs or to decrease the incidence of unwanted side effects caused by NSAIDs, especially those related to GI and renal toxicity.

Among better evaluated combinations are NSAIDs and opiates; they are frequently used in the treatment of postoperative pain and in cancer, in order to reduce the required dose of opiates. Other combinations included NSAIDs and α_2-adrenergic agonists, steroids, copper or others. In some occasions a combination of drugs had, as the main goal, an improvement of the therapeutic effects and the reduction of NSAID-induced side effects. On other occasions, however, the aim was to reduce the dose required with other drugs, for instance, opiates, in order to diminish their unwanted side effects, i.e. tolerance, dependence, respiratory depression, etc.

NSAIDs and Opiates

The combined administration of opiates and COX inhibitors is being used clinically to lower dosage of opiates, and, therefore, to reduce the incidence of adverse effects produced by them (23). This therapy seems to be effective in the treatment of neuropathic pain, an important and rather frequent problem that lacks effective pharmacological solutions, and one that severely reduces the quality of life in many patients.

In a randomized, double-blind, study in patients suffering from upper abdominal surgery, the patients who received ketorolac required less morphine than the control group, showing a useful morphine sparing effect caused by this NSAID (56). A similar interaction was observed with indomethacin and opiates in patients after major abdominal surgery (108). In this study, the duration of the requirement for morphine, as well as the intensity of pain scored after surgery, were significantly lower in patients who received morphine and indomethacin than in those receiving morphine and placebo. The enhancement of the analgesic efficacy of fentanyl by the administration of diclofenac was also tested in patients that had a total hip replacement. Patients, who received diclofenac before the administration of the opiate, showed a significant reduction in the amount of fentanyl needed in comparison to the placebo group (81). Similar interactions have been observed between ketorolac and morphine in the relief of post-Cesarean pain (127), as well as in cancer pain (18), with no enhancement of the adverse effects of morphine.

In animal studies, the nociceptive responses induced by nerve ligation were little affected by the separate administration of morphine or ketorolac. However, when the animals were treated with both compounds together the nociceptive responses were greatly reduced, and a synergistic effect was observed (80). In dopamine neurons involved in mechanisms of rewarding, the administration of morphine in rats pretreated with the COX inhibitors nimesulide or indomethacin induced a potent increase of the firing rate. It was concluded that COX inhibitors enhanced the rewarding actions of morphine in rats (96). Also, the administration of indomethacin enhanced the inhibitory actions of opioids in periaqueductal grey neurons, which are involved in the processing of nociceptive information. This effect seems to be due to a mechanism that involves the modulation of gamma amino butyric acid (GABA) presynaptic receptors and it seems to be due to the inhibition of the COX-1 rather than the COX-2 enzyme (130).

A potent synergy was observed between ketorolac and morphine in the formalin test, but an interaction between COX inhibitors and kappa opioids was only additive (85). Also, the pretreatment of non-antinociceptive doses of ketorolac produced a significant enhancement of the morphine effect in rat nociceptive responses to noxious colorectal distension (90). In withdrawal reflexes recorded as single motor units in anesthetized rats, we observed that the potency of the antinociceptive activity produced by the μ-opioid re-

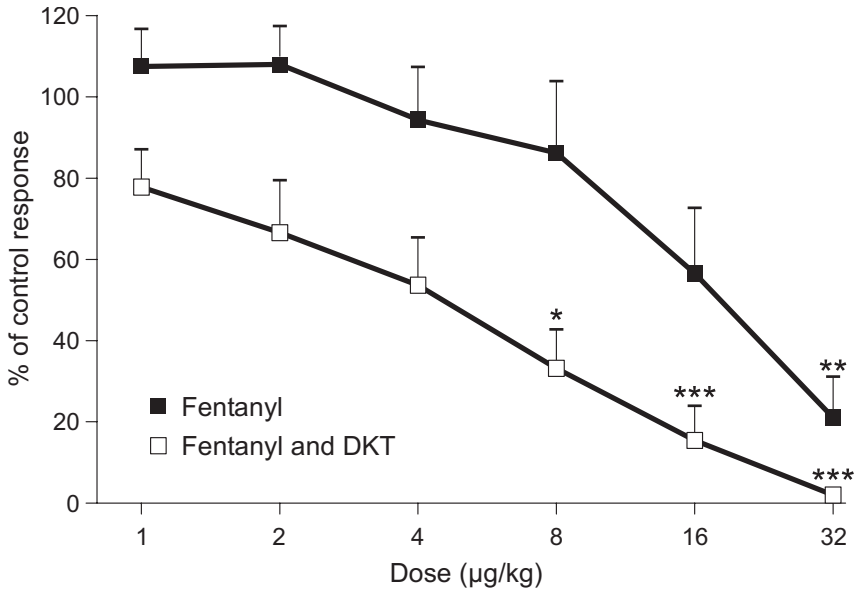


Fig. 8. The combined administration of NSAIDs and opiates results on some occasions in an enhancement of the antinociceptive activity greater than that observed when the compounds are administered separately. The figure shows an example of the antinociceptive effect of the μ-opioid receptor agonist fentanyl that was significantly enhanced in the presence of non-analgesic doses of dexketoprofen trometamol (data taken from ref. 55).

ceptor agonist fentanyl was enhanced fivefold when tested in the presence of less than effective doses of the NSAID dexketoprofen trometamol (Fig. 8). Furthermore, the duration of the analgesic effect of fentanyl, which is very short after systemic administration, was prolonged at least twofold by dexketoprofen trometamol (55).

The actual mechanism of interaction between opiates (mainly μ-opiates) and COX inhibitors in nociception is not clear. A reversal effect of the antinociceptive actions resulting from the combined administration of NSAIDs and opiates has been observed on some occasions after the administration of the opioid receptor antagonist naloxone (85,90). This indicates a modulatory effect on the opioid receptor or, at least, that the final antinociceptive action is mediated by opioid receptors. However, other studies showed a lack of reversion after the administration of high doses of the opioid receptor antagonist naloxone (55 and references within) and that antinociceptive actions of COX inhibitors are, in most cases, not reversed by opioid receptor antagonists (85,92). These data suggest a mechanism independent of a direct modulation of opioid receptors. Several hypotheses on possible mechanisms of action have been considered (85). The mechanism of interaction between COX inhibitors and opiates might not be unique, and different interactions might take place depending on the type of compounds used or even the experimental protocol or type of preparation. In experiments carried out on rat withdrawal reflexes for example, the antinociceptive activity of the NSAID flunixin was reversed and prevented by the administration of the opioid receptor antagonist naloxone, but only in some specific situations, as for instance in spinalized preparations (67). Vaughan et al. (131) suggested an interesting mechanism that would explain the synergistic interaction between opioids

and COX inhibitors in periaqueductal grey neurons. According to them, the inhibition of COX activity by NSAIDs would divert the metabolism of the arachidonic acid through the lipoxygenase pathway. The activation of the μ-opioid receptor would also cause an activation of phospholipase A_2 giving place to the production of more lipoxygenase metabolites than in other situations (since COX activity is blocked). These metabolites, in turn, would induce pain relief probably by activation of a voltage-dependent potassium conductance that decreases the duration of action potentials and inhibits the release of GABA. This hypothesis is also supported by other studies that have shown an activation of voltage-dependent potassium conductances by opioid receptor agonists (146). In any case, the mechanism of interaction between opiates and NSAIDs is far from clear in most circumstances; other possibilities that include glutamate receptors or NO synthesis have to be considered.

OTHER COMBINATIONS OF DRUGS

NSAIDs and α_2-Adrenergic Agonists

In patients suffering from low back pain, combined administration of ibuprofen and the α_2-adrenergic agonist tizanidine produced a more pronounced analgesia and fewer side effects, than with either drug alone (14). In patients with acute back, neck or shoulder pain diclofenac was effective, but the combined treatment with diclofenac and tizanidine was more efficacious in almost all parameters studied and produced fewer adverse effects than monotherapy with diclofenac (45, and references within).

The enhancement of antinociceptive activity of COX inhibitors by α_2-adrenergic agonists has also been observed in studies performed in animals. For instance, the administration of intrathecal ketorolac combined with the α_2-adrenergic agonist ST-91 caused a synergistic antinociceptive effect in the rat formalin test (85). In addition, the α_2-adrenergic agonists tizanidine and clonidine significantly enhanced the antinociceptive and anti-inflammatory effects of the COX inhibitors nimesulide, meloxicam or naproxen in rodents, and reduced their ulcerogenic effects (73). By intrathecal administration to rats the mixture of clonidine and ketoprofen produced only additive effect, but combined intraperitoneal (i.p.) administration of clonidine and ketoprofen, produced an antinociceptive effect with potency suggesting supra-additivity (106). These results indicate that the enhancement of antinociceptive effect might be mediated preferentially by supraspinal interaction, since by i.p. administration the drugs would be expected to distribute to the spinal cord as well as to the supraspinal sites.

We concluded that some COX inhibitors might have antinociceptive activity which is, in addition to COX inhibition, modulated by adrenergic systems, probably at supraspinal levels. The combined administration of NSAIDs and α_2-adrenergic blocking drugs may, therefore, constitute a useful approach to the treatment of chronic pain.

Cu-NSAIDs

There is evidence that copper is involved in the mechanism(s) responsible for the generation and/or maintenance of inflammation. The actual role of copper in inflammation is, however, not well understood. The administration of copper causes an anti-inflammatory

effect, whereas the concentration of copper in plasma increases during inflammation, and so, it is not clear whether copper can be considered a pro- or an anti-inflammatory agent (15). However, it seems evident that some substances have anti-inflammatory properties when administered as copper complexes, and some NSAIDs seem to be more effective than their parent compounds when combined with copper (95 and reference within). The mechanisms underlying this effect are not clear. Some experiments have led to the conclusion that the administration of copper-NSAIDs might result in an increase of Cu(II)-histidine complexes, that might, in turn, cause a protection against hydroxyl radicals (95). Other authors have proposed that the absorption of copper is facilitated in the GI mucosa by NSAIDs, due to their lipophilic nature (123). The possible anti-inflammatory activity of copper would therefore contribute to the final enhancement of the effect (123). The amount of copper in Cu-NSAIDs complexes, however, does not seem to correlate with the observed anti-inflammatory activity. The salicylate complex of Cu(II), for example, was about 30 times more effective as an anti-inflammatory agent than aspirin when given alone (124) and showed greater anti-inflammatory activity than aspirin in rats (84). Other studies found no real advantage in the use of these compounds (140, and references within).

No large clinical data on the analgesic and anti-inflammatory effects of Cu-NSAIDs, are available. There is also not much information available on the effect of Cu complexes on the NSAID-related adverse effects. In animal experiments they seem to cause fewer gastric adverse effects than their parent NSAIDs (140) It is not yet clear, however, whether in humans they cause any protection from side effects of NSAIDs. It is premature to infer an actual therapeutic advantage in the treatment of pain and inflammation with Cu-NSAIDs. Future studies are needed to elucidate the therapeutic and the adverse effects of these complexes in animals as well as in humans.

NSAIDs and Steroids

Glucocorticosteroids are effective drugs in the treatment of chronic inflammation. The mechanism of action of these compounds is the inhibition of inflammatory mediators derived from the COX and lipooxygenase pathways. As for NSAIDs, the use of steroids is quite limited in many cases, because of their adverse effects, especially severe in some patients and in long-term treatment. A therapy based on the co-administration of low doses of steroids and NSAIDs has been studied as another attempt to minimize the unwanted side effects produced by these drugs and to enhance their analgesic and anti-inflammatory efficacy. In fact, supra-additive anti-inflammatory effects have been observed in rats with the combination of diclofenac and dexamethasone (5). When dexamethasone or diclofenac were given separately at low doses, they caused very little effect on carrageenan-evoked spinal c-Fos expression and the associated edema in rats. However, when these compounds were co-administered at low doses to rats, they strongly reduced carrageenan-evoked peripheral edema and c-Fos protein-like immunoreactivity in dorsal horn neurons (24). The effect was twice the sum of the effects produced by the two drugs given separately. The combinations of steroids with NSAIDs appear, therefore, to be useful in the treatment of pain and inflammation. However, the number of studies performed in animals and humans is still low, and the available data are not conclusive.

NSAIDs and Caffeine

Controversial effects have also been observed with the combined administration of NSAIDs and caffeine in the treatment of pain, both in animal experiments and in clinical studies. While some studies show that caffeine induced a clear enhancement of the analgesic effects of NSAIDs (61), other studies showed that a combination of NSAIDs with caffeine is ineffective (97) or that caffeine decreased the effect of some COX inhibitors (119). It appears, therefore, that caffeine may possibly be effective in the enhancement of therapeutic activity of NSAIDs, but only under certain conditions, in certain pain states and at specific dose ratios (61).

CONCLUSIONS

In summary, new COX inhibitors are very valuable and promising therapeutic agents that open a wide range of possibilities in the treatment of moderate to high intensity pain, including neuropathic pain. COX-2 selective inhibitors may be of use in many diseases, especially in chronic situations in which there is no need for cardiovascular protection. The dosage used, though, seems to be crucial and more studies are needed to clearly separate their efficacy and toxicity. Other non-selective COX inhibitors, like the NO-donors or the tromethamine salt derivatives of classic NSAIDs, have to be seriously considered as the analgesics of choice by oral administration. They exhibit not only a very high efficacy, but also a substantially improved adverse effects profile. They inhibit platelet aggregation, and offer, therefore, cardiovascular protection. The combination of COX inhibitors with other drugs, e.g., opiates, might be very useful, not only as analgesic, but also as opiate sparing therapy. In combination with opiates, COX inhibitors might represent a treatment of choice for cancer pain or for any other disorders that require the long-term use of opiates.

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