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Review

In vitro and in vivo pharmacological evidence of selective cyclooxygenase-2 inhibition by nimesulide: An overview

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Abstract. Most available nonsteroidal anti-inflammatory drugs (NSAIDs) inhibit both the constitutive cyclooxygenase-1 (COX-1) and the inducible cyclooxygenase-2 (COX-2), resulting in inhibition of prostaglandin (PG) and thromboxane (TX) biosynthesis. The inhibition of COX-2 might be the cause of the favourable anti-inflammatory, analgesic and antipyretic effects of NSAIDs, whereas that of COX-1 might result in unwanted gastrointestinal, renal and possibly other side-effects. Nimesulide is a sulfonanilide compound with anti-inflammatory properties. Its pharmacological profile (better inhibition of PG synthesis in inflammatory areas than in gastric mucosa), suggested that it might be a selective inhibitor of COX-2. In several in vitro assays using either purified COX-2 and COX-1 preparations or cell preparations (both from animal and human origins) expressing COX-1 or COX-2, ten out of eleven different groups have demonstrated that nimesulide selectively inhibits COX-2. The COX-2/COX-1 inhibitory ratio varies, according to the assay preparation, from about 0.76 to 0.0004 i.e. a 1.3 to 2,512-fold higher selectivity for COX-2 than for COX-1. Moreover, an in vivo whole blood assay performed on healthy volunteers demonstrated a significant fall in COX-2 PGE_2 production without any effect on COX-1 TXB_2 production in subjects treated with nimesulide (100 mg b.i.d. for 2 weeks) versus no effect on COX-2 PGE_2 and an almost total suppression of COX-1 TXB_2 in subjects treated with aspirin (300 mg t.i.d. for 2 weeks). Nimesulide can thus be considered a relatively selective COX-2 inhibitor. At the recommended dosage of 100 mg b.i.d., it is as effective an analgesic and anti-inflammatory agent as classical NSAIDs, and a well-tolerated drug with few side-effects according to large-scale open studies and a global evaluation of a large number of controlled and non-controlled comparative trials.

Key words: Nimesulide – Cyclooxygenase-2

Introduction

Until recently, one cyclooxygenase (COX) was thought to be responsible for both the physiological production of prostaglandins (PGs) and their increased production when inflammation occurs.

In 1990, however, Masferrer and his group reported that the bacterial lipopolysaccharide (LPS)-induced synthesis of PGs in human monocytes in vitro and in mouse peritoneal macrophages in vivo was inhibited by dexamethasone and associated with de novo synthesis of new COX proteins [1, 2].

About one year later, an inducible COX was identified as a different isoform from the constitutive enzyme. COX-2, the inducible enzyme, is encoded by a different gene from COX-1, the constitutive enzyme. Both enzymes have about 60% homology and demonstrate the same affinity and capacity to convert arachidonic acid (AA) to PGH_2. The amino acid residues thought to be important for this enzymatic conversion are conserved in both structures [3–8].

High levels of COX-1 are expressed in platelets [9], vascular endothelial cells [10], stomach [11, 12], and kidney collecting tubules [11, 13, 14]. COX-1 levels remain constant for the most part, but occasionally small 2- to 4-fold increases are observed following hormonal or growth factor stimulation [15, 16].

COX-2 is almost undetectable in most tissues under physiological conditions, but its expression can be considerably increased (10- to 80-fold) during inflammation or following exposure to mitogenic stimuli [7, 13, 14, 17]. COX-2 expression is induced by growth factors, phorbol esters, interleukin-1 in fibroblasts [3, 11, 18], LPS in monocytes [1] and macrophages [2, 19, 20], inflammation in synoviocytes [21], or ovulation in rat follicles [8]. COX-2 induction is completely inhibited by anti-inflammatory steroids [18, 22, 23] suggesting that its encoding gene belongs to the same family of glucocorticoid-sensitive inflammatory response genes to which the inducible nitric oxide synthase gene (iNOS) also belongs [11, 24, 25].

Because COX-1 is expressed constitutively in most tissues, it was hypothesized that COX-1 might be a 'housekeeping' enzyme whose usual purpose is to produce PGs in response to stimulation by circulating hormones that regulate normal physiological processes. Conversely, COX-2 seems to be responsible for the increased production of PGs associated with the inflammatory process. This is strongly suggested by its induction by a large array of inflammatory mediators and by the abolition of its induction by anti-inflammatory steroids. This also suggests that COX-2 inhibition might be the cause of the therapeutically favourable effects of non-steroidal anti-inflammatory drugs (NSAIDs), whereas COX-1 inhibition might be the source of their unwanted side-effects.

COX-1 and COX-2 inhibition by NSAIDs

In an attempt to understand how NSAIDs interact with each kind of COX, their inhibitory potency was tested on both enzymes and their ratio of selectivity for COX-2 versus COX-1 was established. For the standard NSAIDs that have been tested, the spectrum of activity ranges from a high selectivity towards COX-1 to an equiselectivity for both COX. However, the absolute degree of inhibitory potency and the ratio of selectivity showed significant variation according to the source of the enzyme or the type of cells or tissues used for the assays. For instance, the COX-2/COX-1 selectivity ratio for diclofenac was 0.07 when comparing COX-1 from human platelets with COX-2 from rat mesangial cells, but 2.23 when comparing COX-1 from unstimulated guinea pig peritoneal macrophages with COX-2 from LPS-stimulated guinea pig peritoneal macrophages [24, 26–28]. Nevertheless, despite these large variations, the ranking order of selectivity remains roughly the same from one study to another. The NSAIDs with the highest selectivity for COX-1 and the lowest for COX-2, and those with the lowest selectivity for COX-1 and the highest for COX-2, were apparently the same whatever the preparation used for the assay [24, 26–34].

Thus, using bovine aortic endothelial cells as the source of COX-1 and J-774.2 macropahges as the source of COX-2, Vane and his group [28, 33] found that piroxicam, tolmetin, aspirin, sulindac and indomethacin showed greatest preference for COX-1 with a COX-2/COX-1 ratio of 250, 175, 166, 100 and 60 respectively, and naproxen, diclofenac, meloxicam and flurbiprofen greatest preference for COX-2 with a COX-2/COX-1 ratio of 0.6, 0.7, 0.8 and 1.3, respectively. Using unstimulated guinea pig peritoneal macrophages as the source of COX-1 and LPS-stimulated guinea pig peritoneal macrophages as the source of COX-2, Engelhardt et al. [27] confirmed that meloxicam and diclofenac with COX-2/COX-1 ratios of 0.33 and 2.2 respectively had the best COX-2 selectivity, compared with piroxicam and indomethacin which had the best COX-1 selectivity, with ratios of 33 and 30 respectively.

Quite similar results were obtained in other assays performed with other preparations. Laneuville et al. [30] determined the affinities of 13 NSAIDs for human COX-1 and COX-2 in microsomal suspensions of transfected COS-1 cells and found about the same selectivity ratios as obtained by Vane's group. Indomethacin, piroxicam and phenylbutazone demonstrated the highest COX-1 selectivity, and ketorolac, etodolac and 6-methoxy-2-naphtylacetic acid (6-MNA), the active metabolite of nabumetone, the lowest COX-1 selectivity. Gierse et al. [34] made grossly comparable observations using cloned human COX-1 and COX-2 expressed in Sf-9 insect cells utilizing a baculovirus expression system, and extracted from the microsomal fraction. In this model, however, indomethacin was less evidently COX-1-selective whereas naproxen was much more COX-1-selective.

The existence of two different COX isozymes prompted a search for more selective COX-2 inhibitors. As a first attempt, the already available NSAIDs were tested. Most of them are better inhibitors of COX-1 than COX-2. Some of them, however, appear to be either equipotent inhibitors of both isozymes or even slightly better inhibitors of COX-2, such as etodolac [30], 6-MNA [13, 24, 30, 31], diclofenac [26–28, 30, 33], and (in certain models only) naproxen and flurbiprofen [28, 33] or mefenamic acid and niflumic acid [35]. However, among the NSAIDs already marketed only an oxicam compound, meloxicam [26, 27, 36] and a sulfonanilide compound, nimesulide, are significantly better COX-2 than COX-1 inhibitors.

Animal pharmacological data obtained with nimesulide

Early pharmacological studies performed in the late eighties suggested a more potent inhibitor effect of nimesulide on PG biosynthetic pathways in inflammatory areas than in other tissues. Tofanetti et al. [37] had demonstrated that a single oral administration of nimesulide, in contrast to a single oral administration of indomethacin, decreased PGE_2 and thromboxane (TX) B_2 synthesis more potently in inflammatory exudates caused by carrageenan cotton pellet implants, now known to be a COX-2-dependent synthesis, than in the gastric mucosa, now known to be COX-1-dependent. Their results indicate an average COX-2/COX-1 ratio of 0.08 and were corroborated by Ceserani et al. [38] who demonstrated that in rats, the nimesulide dosage inducing ulceration in 50% of the animals (UD50) was higher than $20\,mg.kg^{-1}$ orally versus $1.6\,mg.kg^{-1}$ orally for indomethacin. Tanaka et al. [39] also showed that nimesulide ($UD50 = 106\,mg.kg^{-1}$) is better tolerated by rats than indomethacin ($UD50 = 2.9\,mg.kg^{-1}$), piroxicam ($UD50 = 6.7\,mg.kg^{-1}$) and ibuprofen ($UD50 = 81\,mg.kg^{-1}$). This confirmed the good gastric tolerance of nimesulide already noted by Rainsford [40] who compared the ulcerogenic activity of 100 mg nimesulide (described under the name of R-805) with that of several other NSAIDs at the same 100 mg dose in rats stressed by cold for 45 min after drug administration, using a lesion index calculated on the basis of the number of rats with lesions, the number of lesions, and their severity 2 h following drug administration. The index for nimesulide, 4.6, was especially low when compared with 9 for ibuprofen, 10.1 for meclofenamic acid, 11.6 for phenylbutazone, 16.8 for flufenamic acid, 18.6 for naproxen, 24.7 for niflumic acid, 36 for flurbiprofen, 42.1 for ketoprofen and 77 for tolmetin. Moreover, compared with aspirin, indomethacin and naproxen, Carr et al. [41] have demonstrated that nimesulide has anti-inflammatory activity in rat adjuvant arthritis at a dosage ($0.2\,mg.kg^{-1}$) far

lower than that associated with gastrointestinal blood loss ($100\,mg.kg^{-1}$).

In a recent abstract, Godessart et al. [42] confirmed this low ulcerogenic profile after acute and subacute treatment in rats. The ulcerogenic potential of three classical NSAIDs (indomethacin, diclofenac and naproxen) was compared with that of nimesulide and the experimental COX-2 inhibitor SC-58125. The therapeutic index for the acute treatment was calculated, comparing the gastric damage induced 6 h after dosing and the inhibition of the carrageenan-induced oedema. The results showed that nimesulide and SC-58125 were the safest compounds as both have a therapeutic index >100.0, whereas indomethacin and diclofenac showed the lowest values (9.4 and 6.0 respectively). Naproxen presented an intermediate therapeutic index value of 56. Similar results were obtained in the subacute treatment studies. The subacute treatment therapeutic index was calculated by comparing the gastric and intestinal injury produced after 4 days of treatment with the anti-inflammatory activity observed in the adjuvant arthritis model after 7 days of treatment. Diclofenac and indomethacin had a low therapeutic index ≤20 whereas nimesulide and SC-58125 had a high therapeutic index >75 and appeared again to be the safest compounds Again, naproxen was less ulcerogenic than indomethacin or diclofenac.

The pharmacological profile of nimesulide in animals suggested that it might be an NSAID with selective inhibitory activity on COX-2 [37–42]. Since 1994, several groups have shown, in vitro [35, 43, 44, 46, 47, 51, 53, 54, 56–58, 64, 66] and in one human in vivo model [65], that nimesulide demonstrates a good selectivity as a COX-2 inhibitor.

In vitro studies and in vivo whole blood assay with nimesulide

Tavares et al. compared the activity of nimesulide with that of indomethacin on prostanoid biosynthesis in fresh gastric mucosa pieces obtained by endoscopy (a few centimetres away from any visible lesion), and on prostanoid biosynthesis in human LPS-stimulated peripheral blood leukocytes [43, 44]. It was assumed that COX-1 would be expressed in gastric mucosa and COX-2 in leukocytes, but tissue expression of COX-1 and COX-2 was not characterized. Other difficulties for the interpretation of the results are the different times of incubation in the assay for inhibition of COX-1 in gastric tissue and COX-2 in leukocytes. So, gastric mucosa pieces and leukocytes were incubated with nimesulide (30 min at 4 °C for gastric mucosa and 1 h at 37 °C for leukocytes). This was followed by a further incubation at 37 °C of 30 min for gastric mucosa and 24 h for leukocytes after LPS (5 μg/ml) stimulation. In gastric tissue, nimesulide was approximately 6- to 22-fold less potent than indomethacin (IC50 for PGE, TXB_2, 6-keto-$PGF_{1\alpha}$:14.8 versus 2.5 μM; 12.8 versus 1.0 μM; 31.1 versus 1.4 μM; $p < 0.05$ to 0.02). In leukocytes, nimesulide was only 1.5 to 5-fold less potent than indomethacin, and as both substances caused more than 50% inhibition at the lowest concentration, an IC50 could be approximated by extrapolation only (0.22 versus 0.15 μM for PGE; 0.93 versus 0.18 μM for TXB_2; 0.42 versus 0.15 μM for 6-keto-$PGF_{1\alpha}$; all $p < 0.05$) [43]. From these data, the estimated COX-2/COX-1 ratio of nimesulide was 0.015 for PGE, 0.073 for TXB_2 and 0.013 for 6-keto-$PGF_{1\alpha}$. Unlike many others [28, 30, 31–33], these authors observed a more selective effect of indomethacin on COX-2 than on COX-1, with ratios varying from 0.06 for PGE to 0.18 for TXB_2 [43]. However, when using COX-1 purified from ram seminal vesicles and COX-2 purified from sheep placenta (incubated for 5 min with the drug alone before a further 2 min incubation in the presence of AA at 37 °C), they found a 6.8 ratio for indomethacin, and were unable to find an inhibitory effect of nimesulide on COX-1 up to 100 μM [44] whereas the IC50 for COX-2 inhibition was 90.3 μM. This is high compared with the Cmax of 10.1 μM obtained at steady state in patients treated for 7 days with the usual nimesulide dose of 100 mg b.i.d. [45]. As COX-2 inhibition by nimesulide was described as a time-dependent mechanism [46], this high IC50 could be explained by the short time of incubation (7 min) performed in this study. Much lower IC50 values were obtained with longer incubation times [46, 47].

Moreover, these observations raise the point of the role of each enzyme [48]. So far, the expression of COX-2 in the gastrointestinal tract of healthy human subjects has not been fully characterized. However, when inflammation is present in the gut, as in *Helicobacter pylori* duodenitis, one might anticipate some increase of COX-2 in this tissue resulting from infiltrating leukocytes and other inflammatory cells [49, 50]. This might explain why the difference in COX-2 selectivity between nimesulide and indomethacin was less pronounced in this assay than in other models.

In a recent abstract, the same authors presented the results of a comparison between nimesulide and six conventional NSAIDs (indomethacin, naproxen, tolmetin, diclofenac, piroxicam and ibuprofen). It appears that nimesulide was the only drug that did not inhibit PG-production by COX-1 from ram seminal vesicles but caused a concentration-related inhibition of COX-2 from sheep placenta (5 min preincubation with the drug with a further 2 min incubation in the presence of AA at 37 °C). All the other NSAIDs caused a concentration-related inhibition of both COX-1 and COX-2 [51].

Taniguchi et al. [47] have also compared the activity of nimesulide with that of indomethacin on purified COX-1 from ram seminal vesicles and purified COX-2 from sheep placenta (10 min preincubation with the drug with a further 6 min incubation in the presence of AA at 25 °C). In this study, the 7.1 μM IC50 obtained for COX-2 inhibition by nimesulide was much lower than the 90.3 μM IC50 found by Tavares et al. in the same in vitro model. This discrepancy is most probably due to the time-dependency of COX-2 (but not of COX-1) inhibition by nimesulide [46], resulting in stronger inhibition after 16 min than after 7 min incubation. The results of Taniguchi et al. are probably more clinically relevant than those of Tavares et al. In an ordinary clinical setting, the enzyme will be exposed for an extended period to the drug. On the other hand, indomethacin itself exerts a time-dependent inhibition on both COX-1 and COX-2 [52]. This explains the very low COX-2/COX-1 ratio of 0.007 for nimesulide versus the high 51 ratio for indomethacin, obtained by Taniguchi et al. Therefore, the relative COX-2 selectivity of nimesulide appears to be much higher than that of indomethacin in this in vitro setting.

The COX-2/COX-1 ratio of 0.05 published by Carabaza et al. in a recent abstract [53] and obtained with purified ram seminal vesicle COX-1 and purified sheep placenta COX-2 (time of incubation not mentioned), was very similar to that obtained by Taniguchi et al. [47]. Moreover, these authors obtained ratios of 0.08, 0.03, 0.014 and 0.004 for SC 58125, L-745337, NS-398 and CGP-28238 respectively, all selective COX-2 inhibitors under development.

Vane and his group [54], used bovine aortic endothelial cells to look at COX-1 activity and LPS-stimulated J-774.2 macrophages for COX-2 activity. They found an 84-fold greater selectivity of nimesulide for COX-2 than for COX-1 (COX-2/COX-1 ratio = 0.012) and a 50-fold greater selectivity of indomethacin for COX-1 than for COX-2 (COX-2/COX-1 ratio = 50). This again results in a much higher selectivity for COX-2 by nimesulide than by indomethacin in this well-validated intact cell model, whose main drawback is the use of animal cells (COX-1) or cell lines (COX-2) instead of intact human cells.

Chan et al. [55] have described whole cell-based COX-1 and COX-2 assays using human U-937 cells which express COX-1, and human osteosarcoma 143 cells which express COX-2. After 15 min preincubation of the cells with the drug, a further 10 min incubation was performed in the presence of AA at 37 °C. By using this assay, Prasit et al. [56] gave a COX-2/COX-1 ratio < 0.001 for nimesulide. This ratio was similar to that of flosulide and lower than the 0.01 ratio of DuP 697, both drugs under development as selective COX-2 inhibitors, but higher than the <0.0001 of NS-398, another COX-2 inhibitor under development. The difference between nimesulide and indomethacin in COX-2 selectivity observed in this study was of the same order of magnitude as those observed by Taniguchi et al. [47] and Vane's group [54]. The results of Prasit et al. are rather convincing since nimesulide was compared with classical NSAIDs (indomethacin, diclofenac, ibuprofen) and selective COX-2 inhibitors under development (flosulide, NS-398, DuP 697) as references. Nevertheless, possible drawbacks of the model are that cell lines were used and their physiology may be different from 'normal' cells. Moreover, the cell types used are not involved in the inflammatory process.

Barnett et al. [35] compared the activity of several NSAIDs including nimesulide on human COX-1 and COX-2, cloned and expressed in transfected Sf-9 insect cells utilizing a baculovirus expression system, and extracted from the microsomal fraction. Various preincubation times with the drug were tested (0 to 60 min) and followed by a short 45 s incubation with AA at room temperature. Three groups of inhibitors were described: (i) the drugs which inhibit the two isozymes to about the same extent and were classified as equiselective inhibitors (e.g. diclofenac with a 1.7 COX-2/COX-1 ratio), (ii) the drugs that inhibit COX-1 10- to 20-fold more potently than COX-2 (e.g. indomethacin with a 14.7 COX-2/COX-1 ratio) and (iii) the drugs that inhibit COX-2 more than 30-fold more potently than COX-1 (nimesulide with a 0.018 COX-2/COX-1 ratio and NS-398 with a 0.024 ratio, but also mefenamic acid and niflumic acid with 0.03 and 0.006 COX-2/COX-1 ratios respectively). In this model, nimesulide exhibited a clear 55-fold higher selectivity for COX-2. The advantage of this test system is that human (recombinant) enzymes were used. However, the enzymes were not associated with the cell membrane and not glycosylated as in intact cells. Moreover, the surprisingly high COX-2 selectivity of mefenamic acid and niflumic acid, which are not classically described as COX-2 selective inhibitors, might bring into question the predictive value of the model.

In the same model of recombinant human COX-1 and COX-2 expressed in insect cells using a microsomal assay system, Churchill et al. [57] found a COX-2/COX-1 ratio of 0.19 only for nimesulide versus 0.013 for meloxicam at room temperature (20 min incubation before the addition of AA). The same authors, using recombinant human COX-1 and COX-2 expressed in stable transfected COS cells using a whole cell assay, described a COX-2/COX-1 ratio of 0.22 for nimesulide versus 0.07 for meloxicam (30 min incubation followed by 1 h incubation with AA). Diclofenac ratios were 0.52 and 0.38 in the microsomal and whole cell assay respectively, whereas other NSAIDs tested in both assays were better COX-1 than COX-2 inhibitors (piroxicam was tested in the whole cell assay only). In this model, human recombinant enzymes were used in a microsomal assay or in a whole cell assay. The advantage of this system is that human enzymes are used and that, in contrast to their use in Barnett et al. study [35], they are also associated with a cell membrane. A possible drawback, however, is that the conditions used may be different from those of intact cells (even in the whole cell assay, cells are transfected with the enzymes). In the two test systems of Churchill et al., the COX-2 selectivity of nimesulide was slightly higher than that of diclofenac and slightly lower than that of meloxicam.

The model of unstimulated guinea pig peritoneal macrophages for COX-1 and LPS-stimulated guinea pig peritoneal macrophages for COX-2 has frequently been used for testing the COX-2 selectivity of classical NSAIDs including meloxicam but not nimesulide [24, 26–28]. Its main drawback was the use of non-human cells. In their recent abstract, Carabaza et al. [53] presented results from human polymorphonuclear cells as a source of COX-1, and LPS-stimulated human monocytes as a source of COX-2. In this human intact cell model, they found a COX-2/COX-1 ratio of 0.07 for nimesulide versus 0.024, 0.005, 0.004 and 0.003 respectively for the experimental NS-398, CGP-28238, L-745337 and SC-58125 as reference selective COX-2 inhibitors. Despite the high COX-2 selectivity of these experimental drugs compared with that of nimesulide, the ED30 in reducing carrageenan rat paw oedema was actually the lowest for nimesulide (1.9 $mg.kg^{-1}$ versus >2.5, 5.2, 5.2 and 10 $mg.kg^{-1}$ respectively for CGP-28238, NS-398, L-745337 and SC-58125).

Grossman et al. [58] compared 19 NSAIDs for their inhibitory potency on COX-1 dependent PG-synthesis in platelets and COX-2 dependent PG-synthesis in mononuclear cells isolated from human blood. Drugs were preincubated with the platelets or the cells for 1 h followed by a further 10 min incubation with AA at 37 °C. The most selective drugs for COX-2 were nimesulide, diclofenac, and DuP 697, with COX-2/COX-1 ratios of 0.0004, 0.0004 and 0.0003, respectively. Moreover, in this assay, even the less COX-2 selective NSAID, ketoprofen, was twice as potent towards COX-2 than COX-1. It appears from their results that, whilst NSAID IC50 values obtained in platelets fall within 1 log of values obtained when using cloned human COX-1, IC50 values obtained in mononuclear cells differ by

up to 3.5 log units from values obtained by using cloned human COX-2, with all drugs consistently appearing more potent in the mononuclear cells which are a main target for treatment of inflammation. In this test system, nimesulide evidently exhibits a high level of selectivity for COX-2. The advantage of the system is that human cells were used. However, the values obtained with references NSAIDs are significantly different from those obtained by others in the 'human whole blood assay' [59–62]. In this human whole blood assay, as in Grossman's model, human platelets are used to test COX-1 activity and human monocytes to test for COX-2 activity. However, the platelets and mononuclear cells are isolated from whole blood. As a consequence, the binding of the drug to plasma protein is not taken into account in Grossman's model as it is in the whole blood assay. Furthermore, the isolation procedure may modify cell physiology (i.e. membrane permeability). The fact that diclofenac shows a COX-2 selectivity similar to that of nimesulide and better than that of NS-398 and flosulide undermines the validity of the model.

Using their whole blood assay, a model suitable for evaluating the extent of COX-1 and COX-2 inhibition both in vitro and after oral dosing of NSAIDs in humans [63], Patrignani et al. were recently able to present [64] some results obtained in vitro with several conventional NSAIDs and a few COX-2 selective inhibitors. They also showed ex vivo results after oral dosing of human subjects with nabumetone (500 and 1000 mg) and nimesulide (100 mg). Heparinized whole blood samples were incubated with LPS ($10\,\mu g.ml^{-1}$) for 24 h at 37 °C and PGE_2 was measured in plasma. The production of TXB_2 during whole blood clotting for 1 h at 37 °C was evaluated as an index of platelet COX-1 activity. Various NSAIDs i.e. ibuprofen, naproxen, S-ketoprofen, 6-MNA, flurbiprofen, indomethacin and piroxicam, with different gastrointestinal toxicities, were equipotent towards the two isozymes in vitro. Meloxicam and nimesulide were approximately 10-fold while SC-58125, NS-398 and L-745.337 were more than 150-fold more potent towards COX-2 than COX-1. Nabumetone was equipotent in suppressing COX-1 and COX-2 activities in human whole blood ex vivo.

In contrast, ex vivo measurements showed that oral dosing with nimesulide completely suppressed COX-2 activity while partially reduced (by 50%) COX-1 activity. This was confirmed recently by Cullen et al. [65] in 20 healthy volunteers who received either nimesulide 100 mg b.i.d. ($n = 10$) or aspirin 300 mg t.i.d. ($n = 10$) for 14 days. They found no effect of aspirin on COX-2 as estimated by the endotoxin-stimulated generation of PGE_2 in whole blood, whereas PGE_2 formation fell to about 10% of its basal value in the nimesulide-treated subjects, recovering 24 h after nimesulide discontinuation. Nimesulide had no effect on COX-1 as estimated by determination of serum TXB_2 whereas TXB_2 production was almost totally suppressed by aspirin (98% inhibition). Moreover, no changes were detected in urinary prostanoids (TXB_2 and 6-keto-$PGF_{1\alpha}$) generated by renal COX-1 in subjects treated with nimesulide.

Finally, using mouse recombinant enzymes (10 to 20 min incubation before a further 10 min incubation with AA), Huff et al. [66] found almost no selectivity for nimesulide (a COX-2/COX-1 ratio of 0.76) whereas NS-398 exhibited a ratio of <0.0016. A clear drawback of this test system, however, is that mouse enzymes were used instead of the human forms.

It appears from all these data that nimesulide, with a COX-2/COX-1 ratio varying according to the assay preparation, from about 0.76 [66] to 0.0004 [58] (a 1.3- to 2,512-fold higher selectivity towards COX-2 than COX-1) can be considered as a relatively selective COX-2 inhibitor.

Chemistry of selective COX-2 inhibitors

The structural types found to be most selective apparently came from two main series of compounds known for 20 years or more: the sulfonamides and the non-acidic tricyclics.

Nimesulide is the only non-experimental compound of the sulfonamide series. NS-398 [67–70], flosulide or CGP-28238 [71, 72], FK-3311 [73], L-745337 [56, 74] or MK-966, and T-614 [75] are nimesulide analogues, all exhibiting COX-2 selectivity in vitro and/or in preclinical studies. However, most of these compounds are presently not in clinical development. Flosulide was in Phase III clinical trials in the early nineties when its clinical development was interrupted because of renal side-effects [76]; MK-966 is in early clinical development and has shown analgesic activity in patients with osteoarthritis (OA) and following dental extraction, as well as lack of gastric toxicity in a 7 day gastroscopy study comparing MK-966 to placebo, ibuprofen and aspirin [77].

The non-acidic tricyclic group has a structure consisting of two phenyl groups attached ortho to a 5 member ring. The first compound of this group which was demonstrated to possess anti-inflammatory properties by Tanaka et al. [78] in the early seventies was bimetopyrol, but its COX-2/COX-1 ratio is unknown. DuP-697 [24, 34, 52, 79], SC-58125 and SC-57666 [12, 14, 73, 80, 81] belong to this series, and they all demonstrate a high selectivity for COX-2. SC-58125 and SC-57666 are 1400 and 3800-fold more selective towards COX-2 than COX-1, respectively [73]. Again, all these compounds are in preclinical development only. Celecoxib (SC-58635) is currently in clinical development for the treatment of the signs and symptoms of arthritis and pain, and has shown an improved safety profile compared to other NSAIDs in these conditions [82].

From a chemical point of view, it appears that all these selective COX-2 inhibitors possess a methylsulfonyl group which seems important for the selectivity.

Time-dependent effects of nimesulide and other COX-2 inhibitors

Since Vane's first description of COX inhibition by aspirin in 1971 [83], all NSAIDs used in the clinics have been shown to possess this property whatever the preparation or the enzyme source chosen for the assay [84]. It was also shown that not all NSAIDs react in the same way with the PG-synthase enzymatic complex. Three different classes of NSAIDs have been defined according to their mode of inhibition of COX [30, 85].

Class I NSAIDs compete reversibly with AA for binding to COX active sites. They are simple competitive inhibitors and form an enzyme-inhibitor (EI) complex rapidly and

reversibly. Piroxicam [86], ibuprofen [30, 31, 87], mefenamic acid [87], and sulindac sulfide [31] belong to this class [85].

Class II NSAIDs have a more elaborate mechanism of action. The quick formation of a reversible EI-complex results in COX-protein structural changes reflecting the formation of a secondary semistable EI-complex after the initial binding of the NSAID. This formation, however, does not involve covalent modification of the enzyme. When the drug is removed, the enzyme only slowly regained activity. The rate of formation of the secondary semistable EI-complex, and the rate of recovery of the enzymatic activity after drug removal, vary from one NSAID to another [30, 85, 87–90]. Indomethacin, meclofenamic acid and flurbiprofen are members of this class [30, 85, 89].

Class III NSAIDs form an irreversible complex with the enzyme by covalent modification of the COX-protein, which never recovers its enzymatic activity. Aspirin is the only NSAID belonging to this class [87, 91, 92].

These different modes of binding to the enzyme explain why COX inhibition by class I NSAIDs does not significantly change with incubation time, whereas inhibition by class II NSAIDs appears to be time-dependent [30, 85, 89].

Several studies have recently been performed with selective COX-2 inhibitors, including nimesulide, in order to determine the time-dependency of their interaction with COX-1 and COX-2. Thus, it was shown that both DuP-697 and NS-398 are time-dependent inhibitors of COX-2 but simple competitive inhibitors of COX-1 [93]. This distinction between COX-1 and COX-2 inhibition mechanisms appears to be the basis of the COX-2 selectivity of these drugs [68, 69, 94]. The initial bindings of these compounds to COX-1 and COX-2 are similar but the EI-complex formed with COX-2 (but not with COX-1) results in the formation of a semistable EI-complex. Drugs with selective COX-2 properties seem thus to behave like class I NSAIDs with COX-1 and class II NSAIDs with COX-2 [85]. In contrast, conventional class II NSAIDs have a class II mode of action on both enzymes, or even, with flurbiprofen for example, a class I behaviour with COX-2 and a moderate class II behaviour with COX-1 [30].

Vago et al. [46] have clearly demonstrated the time-dependency of COX-2 inhibition in the presence of nimesulide, i.e. a class II behaviour of nimesulide on COX-2, resulting in a maximum efficacy after 10 to 15 min preincubation with the drug. Nimesulide does not show time-dependency of COX-1 inhibition, i.e. it has class I behaviour on COX-1. This time-dependency is responsible for dramatic modifications of the COX-2/COX-1 inhibitory ratio according to the preincubation time with nimesulide. It varies from <0.7 after 2 min to <0.03, 0.0005 and 0.0007 after 5, 10 and 15 min, respectively. The preincubation times were quite variable in the different studies performed with nimesulide, varying from 5 min in the studies of Tavares et al. [44, 51] to 1 h in that of Grossman et al. [58]. However, in contrast to these observations, Barnett et al. [35] observed time-dependent inhibition both on COX-2 and COX-1 for the selective COX-2 inhibitors NS-398, flosulide and nimesulide. According to their observations, the main difference between selective COX-1 and COX-2 inhibitors lies in the fact that selective COX-2 inhibitors inhibit COX-2 at a much faster rate than COX-1, whereas selective COX-1 inhibitors inhibit COX-1 at a much faster rate than COX-2. Thus, in a clinical setting in which the enzymes are exposed for an extended period to the drug, a COX-2 inhibitor must possess a high degree of in vitro selectivity in order to express this selectivity in vivo.

Clinical relevance of COX-2 inhibition by nimesulide

The greatest anticipated benefit of a COX-2 selective inhibitor is a favourable side-effect profile with no loss of anti-inflammatory activity in comparison with classical NSAIDs.

Efficacy

The efficacy of nimesulide has been well established in several double-blind studies. A dose-response study carried out in about 400 patients suffering from OA in which a placebo group was included has shown the optimal dosage to be 100 mg b.i.d. [95]. Four double-blind short-term (1 to 2 weeks) studies in OA have confirmed the superiority of this dosage of nimesulide over placebo (R. L. Dreiser, personal communication) [96, 97] which was also confirmed in a 3 month double-blind study versus placebo in elderly OA [98]. Five pivotal studies lasting from 3 weeks to 3 months in more than 500 OA patients have demonstrated that nimesulide 100 mg b.i.d. has the same efficacy as piroxicam 20 mg o.d. [97], naproxen 500 mg b.i.d. [99], diclofenac 50 mg t.i.d. [100], ketoprofen 100 mg b.i.d. [97], and etodolac 300 mg b.i.d. [101] and one pivotal study in more than 200 patients suffering from soft tissue rheumatism has demonstrated its equipotency to sodium naproxen 550 mg b.i.d. after two weeks of treatment [102]. This good efficacy was also confirmed in double-blind short-term (3 to 14 days) studies conducted in more than 800 patients suffering from post-traumatic and/or post-surgical conditions [45].

Tolerability

In some placebo-controlled studies, the incidence of side-effects was similar in placebo and nimesulide (100 mg b.i.d.) recipients [95, 97, 98], but globally there were more side-effects with nimesulide than with placebo (R. L. Dreiser, personal communication) [96]. In most studies in which nimesulide (100 mg b.i.d.) was compared with classical NSAIDs, the side-effects (mainly gastro-intestinal) observed with nimesulide were not significantly different than those (also mainly gastro-intestinal) observed with the anti-inflammatory comparator. This was probably due to the short-term duration of these trials and low number of patients included, resulting in too low a number of side-effects.

Recently however, nimesulide's long-term efficacy (100 mg b.i.d.) and tolerability were evaluated in a double-blind study with diclofenac (50 mg t.i.d.) in 279 patients with OA over a 6 month period. A statistically significant 10.9% decrease in drug-related gastrointestinal side-effects was observed in the nimesulide compared to the diclofenac-treated patients [103].

An evaluation of the side-effects reported in clinical trials performed up to 1991 has shown an 8.87% incidence of

side-effects, mainly gastrointestinal, in the 4,224 patients who received nimesulide for various inflammatory and/or painful conditions versus a 16.70% incidence in the 1,017 patients who received the comparative treatments [103]. The latter incidence seems in accordance with data from the literature concerning the frequency of side-effects reported in NSAID recipients [104–106].

The results of this evaluation are corroborated by those of large multicentre open studies. A study performed in general practice setting on 22,938 OA patients demonstrated a good tolerability of nimesulide 100 to 400 mg (mostly 200 mg) per day for 5 to 21 (mean 12) days. Only 8.2% of the patients experienced side-effects, mainly gastrointestinal, a very similar incidence to that reported in the clinical evaluation [107]. A second multicentre open study performed both in general practice and orthopaedic settings in 12,607 patients suffering from acute musculoskeletal injuries (200 mg b.i.d. for 4 days followed by 100 mg b.i.d. up to day 21 maximum) confirmed these results with an even lower 6.8% incidence of side-effects, again mainly gastrointestinal [108]. Moreover, when considering the 8,354 patients aged over 60 years in these two studies, a population more prone to develop NSAID adverse reactions, the 8.9% incidence of side-effects was not higher than in the whole population [109].

The gastric tolerance of nimesulide 100 mg b.i.d. (and of 200 mg b.i.d. in the study versus placebo) was also tested in three endoscopic studies that revealed no difference after one week treatment with placebo in the first study [110], a lower incidence of gastric erosions than with indomethacin (50 mg t.i.d.) after a 12 to 15 day treatment in the second study [111], and the same incidence of gastric erosions as with diclofenac (50 mg t.i.d.) after one month's treatment in the third study [100].

Conclusion

From all the in vitro and in vivo pharmacological animal data performed, it appears that nimesulide is a relatively selective COX-2 inhibitor. It belongs to the sulfonamide family in which several other compounds structurally related to it have also been demonstrated to be selective COX-2 inhibitors. It possesses a methylsulfonyl group which seems to be the hallmark of this selectivity. It also possesses the same time-dependent inhibitory profile as other COX-2 selective inhibitors. In the clinics, at the recommended dosage of 100 mg b.i.d., it is as efficient an analgesic and anti-inflammatory agent as the classical NSAIDs with which it was compared. Moreover, it seems to be well-tolerated with few, mainly gastrointestinal, side-effects according to large scale open evaluations and a global evaluation of a large number of controlled and non-controlled comparative trials performed up to 1991.

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