

Activity of Nimesulide on Constitutive and Inducible Cyclooxygenases

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Summary

Prostaglandins are pro-inflammatory but are gastroprotective. The gastric mucosa synthesizes prostaglandins mainly via constitutive cyclooxygenase (COX-1), whereas leucocytes have inducible enzyme (COX-2). Nimesulide (CAS 51803-78-2) differentially inhibited prostanoid synthesis in these human tissues as well as with *in vitro* enzyme assays, and was less potent than indomethacin (CAS 53-86-1) on COX-1.

Fresh human gastric mucosa was cut finely, washed and pre-incubated (100 mg in 1 ml phosphate buffered saline pH 7.4) with or without nimesulide or indomethacin (0.1–100 µg/ml; 0 °C; 30 min). The fluid was replaced with fresh identical solution, incubated (37 °C; 30 min) and the solution assayed.

Isolated leucocytes from human peripheral blood were incubated ($1-1.5 \times 10^6$, 2 ml Krebs' solution) with or without nimesulide or indomethacin (0.1–100 µg/ml; 37 °C; 1 h), stimulated with lipopolysaccharide (5 µg/ml), further incubated for 24 h at 37 °C and the medium assayed for the prostanoids PGE, TXB₂, 6-keto-PGF_{1α} and the leukotriene LTB₄ by radioimmunoassay (RIA). *In vitro* assays with COX-1 from ram seminal vesicles, or COX-2 from sheep placenta, were performed by pre-incubating the enzymes with vehicle alone (controls) or with drug for 5 min at 37 °C. Arachidonate (10 µmol/l) was added and further incubated for 2 min at 37 °C. Reactions were terminated and PGE determined by RIA.

Both drugs caused concentration-related inhibitions of prostanoid accumulation in incubates of both tissues. Nimesulide reduced PGE accumulation more potently in incubates of stimulated leucocytes than of gastric mucosa. With gastric tissue, nimesulide was less potent than indomethacin by approximately 6–22 fold (IC₅₀ for PGE, TXB₂, 6-keto-PGF_{1α}, respectively; 14.8 vs 2.5; 12.8 vs 1.0; 31.1 vs 1.4 µmol/l; $p < 0.05$ to 0.02). With leucocytes, the concentrations of both drugs, particularly indomethacin, were not low enough to calculate the IC₅₀.

With the *in vitro* assay, nimesulide (0.01 to 100 µmol/l) did not inhibit PGE formation by COX-1 but caused a concentration-related inhibition of PGE formation by COX-2 (4–60%).

These results are consistent with the effective analgesic/anti-inflammatory activity of nimesulide coupled with better gastric tolerance compared to indomethacin.

Zusammenfassung

Wirkung von Nimesulid auf konstitutive und induzierbare Zyklooxygenasen

Prostaglandine sind entzündungsfördernd, haben jedoch eine magenprotektive Wirkung. Die Magenschleimhaut synthetisiert Prostaglandine hauptsächlich mit Hilfe von

konstitutiver Zyklooxygenase (COX-1), während bei Leukozyten induzierbares Enzym vorliegt (COX-2). In der vorliegenden Arbeit wird dargelegt, daß Nimesulid (CAS 51803-78-2) sowohl bei diesen menschlichen Gewebearten als auch bei *In-vitro*-Enzymassays die Prostaglandin-Synthese unterschiedlich stark hemmt und bei COX-1 eine schwächere Wirkung als Indometacin aufwies.

Frische Magenschleimhaut vom Menschen wurde fein geschnitten, gewaschen und (100 mg in 1 ml Salzlösung, die mit Phosphatpuffer auf pH 7,4 eingestellt worden war) mit oder ohne Nimesulid oder Indometacin (0,1–100 µg/ml; 0 °C; 30 min) vorinkubiert. Die Flüssigkeit wurde durch frische Lösung derselben Zusammensetzung ersetzt, inkubiert (37 °C; 30 min) und dann die Aktivität bestimmt.

Isolierte Leukozyten aus peripherem menschlichen Blut wurden ($1-1,5 \times 10^6$, 2 ml Krebs-Lösung) mit oder ohne Nimesulid oder Indometacin (0,1–100 µg/ml; 37 °C; 1 h) inkubiert, nach Stimulation mit Lipopolysaccharid (5 µg/ml) weitere 24 h bei 37 °C inkubiert, und das Medium mit Radioimmunoassay (RIA) auf die Prostanoid-PGE, TXB₂, 6-Keto-PGF_{1α} und das Leukotrien LTB₄ untersucht.

Es wurden *In-vitro*-Assays von COX-1 aus Hammelsamenbläschen und von COX-2 aus Schafplazenta durchgeführt, wobei die Enzyme mit dem Vehikel allein (Kontrolle) oder mit dem Arzneimittel 5 min bei 37 °C vorinkubiert wurden. Es wurde Arachidonat (10 µmol/l) zugefügt und 2 min bei 37 °C weiterinkubiert. Die Reaktionen wurden gestoppt und PGE mit RIA bestimmt.

Beide Arzneimittel bewirkten in beiden inkubierten menschlichen Geweben konzentrationsabhängige Hemmung der Prostanoid-Speicherung. Nimesulid verringerte die PGE-Speicherung in stimulierten Leukozyten stärker als in der Magenschleimhaut. Bei Magengewebe war Nimesulid ca. 6 bis 22mal weniger wirksam als Indometacin (Hemmkonzentration HK₅₀ bei PGE, TXB₂, 6-Keto-PGF_{1α} jeweils 14,8 gegenüber 2,5; 12,8 gegenüber 1,0; 31,1 gegenüber 1,4 µmol/l; $p < 0,05$ bis 0,02). Bei Leukozyten waren die Konzentrationen beider Arzneimittel, insbesondere von Indometacin, nicht niedrig genug, um die IC₅₀ zu berechnen.

Beim *In-vitro*-Assay hemmte Nimesulid (0,01 bis 100 µmol/l) die PGE-Bildung bei COX-1 nicht, bewirkte jedoch bei COX-2 eine konzentrationsabhängige Hemmung der PGE-Bildung (4–60%).

Diese Ergebnisse stehen im Einklang mit der wirksamen analgetischen entzündungshemmenden Aktivität von Nimesulid sowie der besseren Magenverträglichkeit im Vergleich zu Indometacin.

Key words: Anti-inflammatories, non-steroidal · Aulin® · CAS 51803-78-2 · Cyclooxygenases · Mesulid® · Nimed® · Nimesulide · Nisulid®

1. Introduction

There are two forms of cyclooxygenases, key enzymes involved in the formation of prostanoids: COX-1 and COX-2 [1]. COX-1 encoded by a 2.8-kilobase mRNA is the constitutive form of the enzyme, and in the stomach is mainly responsible for synthesising prostanoids which help to maintain gastric mucosal integrity by various processes. In contrast, leucocytes contain mainly COX-2 encoded by a 4-kilobase mRNA, which is an inducible enzyme that forms prostanoids involved in pain and inflammation.

Non-steroidal anti-inflammatory drugs are thought to damage the gastric mucosa at least partly by inhibiting prostaglandin synthesis, whereas inhibition of the synthesis at inflamed sites relieves pain and inflammation. The aim of this study is to determine whether the reported better gastric tolerance to nimesulide¹⁾ (CAS 51803-78-2, N-(4-nitro-2-phenoxyphenyl)-methanesulfonamide) compared to indomethacin (CAS 53-86-1) can be explained by different potencies on the synthesis of eicosanoids in stimulated human leucocytes, in human gastric mucosa and in an assay with purified COX-1 and COX-2.

2. Materials and methods

2.1. Human peripheral blood leucocytes

Human peripheral blood leucocytes from 6 normal healthy male volunteers aged 20–35 years were separated using Histopaque 1099 (Sigma-Aldrich Ltd., Poole, UK) [2]. Isolated leucocytes (1–1.5 ml 10^6 cells in 2 ml Krebs' solution) were incubated in 24-well plates at 37 °C with vehicle alone (controls) or with nimesulide or indomethacin (0.1, 1, 10, and 100 µg/ml). After 1 h the leucocytes were stimulated with lipopolysaccharide (Sigma, 10 µg/2 ml) and further incubated for 24 h. Eicosanoids in the culture fluid were measured by radioimmunoassay (RIA).

2.2. Gastric mucosal incubations

Gastric specimens resected for benign or malignant disease were obtained from operations, and samples taken at least 5 cm from any macroscopically visible lesion. The mucosa and submucosa were cut off together, washed in phosphate buffered saline (PBS), cut into pieces of about 4 mm², washed again in PBS, and the excess fluid drained off. Aliquots of 100 mg were incubated for 30 min at 4 °C with nimesulide or indomethacin, 0.1, 1, 10 and 100 µg/ml or vehicle controls, to allow the drugs to equilibrate at their sites of action. The incubation fluid was discarded (along with the prostanoids and lipoxygenase products released from the tissue prior to the drugs exerting their full effects), and replaced with another aliquot of the original solution. After incubation at 37 °C for 30 min the fluid was removed and eicosanoids measured by RIA.

2.3. Cyclooxygenase enzyme assays

Cyclooxygenase enzyme (one unit of either COX-1 from ram seminal vesicles, or COX-2 from sheep placenta, Cayman Chemical Company, Ann Arbor, MI, USA) was suspended in Tris-HCl buffer (1 ml, 100 mmol/l, pH 8) containing hematin (1 µmol/l) and phenol (2 mmol/l) as co-factors. They were pre-incubated with vehicle alone (controls) or with nimesulide or indomethacin (0.01 to 100 µmol/l) for 5 min at 37 °C. Arachidonate (10 µmol/l, Cayman Chemical Company) was added and further incubated for 2 min at 37 °C [4]. Reactions were terminated by placing the tubes on ice and then extracting excess arachidonate with hexane (3 ml × 2). Prostaglandin E (PGE) was determined by RIA.

2.4. Radioimmunoassay

Using suitable dilutions of antisera and tritiated standards, the samples were assayed in duplicate [3]. Assay sensitivities were 10

to 20 pg, and the intra- and inter-assay coefficients of variation ranged from 2.0 to 5.0 %. As the PGE antiserum did not distinguish between PGE₁ and PGE₂, the results are expressed as PGE. The antisera sources and immunological specificities used are previously published [4, 5].

Tritiated prostanoids and leukotrienes were purchased from Amersham International PLC, Little Chalfont (UK) and DuPont (UK) Ltd. (NEN Products), Stevenage (UK) respectively. Scintillation counting was done using a Packard Tricarb 2200 CA (Packard Instrument Company, Meriden, USA) fitted with a Securia programme. The results are expressed as means ± SEM, and analysed by Student's t-test for paired data (2-tailed).

3. Results

3.1. Leucocyte incubations

Human leucocytes incubated in Krebs' solution and stimulated by lipopolysaccharide 5 µg/ml released various eicosanoids. Prostanoid accumulation was reduced by nimesulide or indomethacin 0.1–100 µg/ml in a concentration-related manner (PGE: 74–99 % and 93–99 %, respectively; 6-keto-PGF_{1α} 50–82 % and 80–95 % respectively; thromboxane B₂ (TXB₂) 54–91 % and 91–98 % respectively (Table 1). LTB₄ accumulation was not significantly affected, except for an inhibition at the highest concentration of either drug (100 µg/ml).

3.2. Gastric mucosal incubations

Human gastric mucosal pieces incubated in PBS released prostanoids and leukotrienes. Nimesulide and indomethacin caused a concentration-related inhibition of basal prostanoid release (Table 2). The reductions by indomethacin 0.1–10 µg/ml were: PGE 31–96 %, 6-keto-PGF_{1α} 47–99 %, TXB₂ 31–97 %. With nimesulide 0.1–

Table 1: Effect of nimesulide (NIM) and indomethacin (INDO) on stimulated eicosanoid accumulation in incubates of human leucocytes: % of control.

Drug conc. (µg/ml)	PGE	6-Keto-PGF _{1α}	TXB ₂	LTB ₄
NIM 0.1	26.0 ± 3.6**	49.7 ± 9.0**	45.8 ± 2.8**	108.9 ± 24.0**
1	17.9 ± 4.6**	28.9 ± 3.2**	24.6 ± 4.2**	98.8 ± 12.8**
10	6.0 ± 1.5**	28.6 ± 8.3**	6.7 ± 1.4**	68.4 ± 6.4**
100	1.2 ± 0.6**	11.9 ± 6.2**	8.7 ± 2.8**	20.0 ± 9.9**
INDO 0.1	6.8 ± 3.5**	20.3 ± 4.1**	8.7 ± 2.8**	109.7 ± 36.1**
1	1.1 ± 0.7**	17.5 ± 4.4**	3.8 ± 1.0**	102.4 ± 33.7**
10	0.7 ± 0.6**	12.0 ± 4.2**	2.6 ± 0.8**	112.1 ± 20.8**
100	1.3 ± 0.6**	4.5 ± 3.9**	1.8 ± 0.4**	31.1 ± 16.9**

Results are expressed as mean % of control ± SEM (n = 6). There were 4 replicates for control incubations and 1 for each drug concentration per volunteer, all measured in duplicate. The following amounts of prostanoids accumulated in the medium during the incubations (pg/10⁶ cell/24 h; means ± SEM): PGE 1694 ± 252, 6-keto-PGF_{1α} 417 ± 66; TXB₂ 12960 ± 1083; LTB₄ 157 ± 33. ** p < 0.02.

Table 2: Effect of nimesulide (NIM) and indomethacin (INDO) on basal eicosanoid accumulation in incubates of human gastric mucosal pieces: % of control

Drug conc. (µg/ml)	PGE	6-Keto-PGF _{1α}	TXB ₂	LTB ₄
NIM 0.1	108.7 ± 7.4	93.7 ± 7.6	117.2 ± 12.0	122.0 ± 16.9
1	72.2 ± 9.4*	62.9 ± 13.5	75.5 ± 5.3	105.1 ± 20.1
10	21.2 ± 2.5**	19.4 ± 6.9*	35.1 ± 8.3**	64.4 ± 14.4
100	6.8 ± 0.8**	1.7 ± 0.8*	4.6 ± 1.2**	54.6 ± 10.1*
INDO 0.1	69.1 ± 9.0**	53.0 ± 15.5	69.1 ± 6.3	113.0 ± 24.0
1	30.1 ± 7.9**	20.7 ± 7.5*	26.5 ± 5.9**	82.7 ± 14.2
10	7.9 ± 1.8**	6.8 ± 2.5*	6.7 ± 1.6**	52.8 ± 3.0*
100	3.5 ± 1.4**	0.7 ± 0.5*	2.8 ± 1.3**	6.0 ± 6.0*

Results are expressed as mean % of control ± SEM (n = 6). There were 4 replicates for control incubations and 1 for each drug concentration per tissue, all measured in duplicate. The following amounts of prostanoids accumulated in the medium during basal incubations (pg/mg in 30 min; means ± SEM): PGE 104 ± 18, 6-keto-PGF_{1α} 128 ± 43; TXB₂ 187 ± 34; LTB₄ 7 ± 3. * p < 0.05, ** p < 0.02.

¹⁾ Manufacturer: Helsinn Healthcare SA, Pambio-Noranco, Lugano (Switzerland). Trademarks: Aulin® (I, CH, IRL, P, F), Mesulid® (B, GR, I), Nimes® (P), Nisulid® (CH).

Table 3: Effect of nimesulide (NIM) and indomethacin (INDO) on PGE production from arachidonate (10 µmol/l) by COX-1 and COX-2.

Drug conc. (µmol/l)	COX-1	COX-2
NIM 0.01	159.9 ± 19.3*	116.4 ± 7.9
0.1	154.1 ± 18.2*	95.9 ± 2.9
1.0	152.5 ± 19.0	78.7 ± 4.3
10.0	145.8 ± 16.3	69.0 ± 4.5*
100.0	103.9 ± 13.6	40.0 ± 7.9*
INDO 0.01	119.7 ± 12.4	105.4 ± 4.5
0.1	77.2 ± 9.5	84.3 ± 7.9
1.0	33.8 ± 1.0**	64.7 ± 14.5
10.0	16.1 ± 1.5**	21.1 ± 2.7*
100.0	9.1 ± 1.2**	13.5 ± 1.0*

Results are expressed as mean % of control ± SEM (n = 4). There were 5 replicates for control incubations and 1 for each drug concentration per experiment, all measured in duplicate. The following amounts of PGE accumulated in the medium during the incubations (pg/U in 2 min; means ± SEM): COX-1, 1320 ± 68; COX-2, 2251 ± 173. * p < 0.05, ** p < 0.02.

10 µg/ml the mean reductions were PGE 0–92 %, 6-keto-PGF_{1α} 47–93 %, and TXB₂ 30–93 %. LTB₄ release was inhibited by indomethacin and, to some extent, by nimesulide.

3.3. Cyclooxygenase enzyme assays

COX-1 and COX-2 (1 unit in 1 ml) when incubated with arachidonate (10 µmol/l) and cofactors produced PGE which was assayed by RIA. Nimesulide (0.01 to 100 µmol/l) did not inhibit PGE formation by COX-1, and lower concentrations (0.01, 0.1 µmol/l) actually increased PGE production (Table 3). Indomethacin caused a concentration-related inhibition of PGE formation.

Nimesulide and indomethacin (0.1 to 100 µmol/l) caused a concentration-related inhibition of PGE formation by COX-2 (4–60 % and 15–87 %, respectively, Table 3).

4. Discussion

Incubates of human peripheral blood leucocytes stimulated by lipopolysaccharide in culture contained PGE, 6-keto-PGF_{1α}, TXB₂ and LTB₄, in agreement with previous findings [7]. Indomethacin was more potent than nimesulide in inhibiting prostanoid synthesis by human peripheral blood leucocytes (overall comparison p < 0.001). However, the IC₅₀ values for the drugs could not be calculated since at the lowest concentration (0.1 µg/ml) they both caused > 50 % inhibition (Table 4).

In incubates of human gastric mucosa, nimesulide and indomethacin each caused a concentration-related inhibition of prostanoid accumulation. Indomethacin was more potent than nimesulide in reducing gastric prostanoid synthesis (overall comparison p < 0.001). The IC₅₀ values for the gastric mucosa were: PGE, 14.8 µmol/l and 2.5 µmol/l; 6-keto-PGF_{1α}, 12.8 µmol/l and 1.0 µmol/l; TXB₂, 31.1 µmol/l and 1.4 µmol/l, respectively, for nimesulide and indomethacin.

Compared with gastric mucosa, inhibition of leucocyte PGE synthesis occurred at lower concentrations of nimesulide and indomethacin.

Using the purified enzyme assay in vitro, nimesulide (0.01 to 100 µmol/l) did not inhibit PGE production by COX-1 (ram seminal vesicles) but did inhibit COX-2 (sheep placenta) with an IC₅₀ of 90.3 µmol/l, while indomethacin inhibited both COX-1 and COX-2 (IC₅₀, 0.6 and 4.1 µmol/l, respectively).

Table 4: IC₅₀ values (µmol/l) of nimesulide and indomethacin.

Gastric mucosal pieces	PGE	6-Keto-PGF _{1α}	TXB ₂
Nimesulide	14.8 ± 2.7	12.8 ± 5.2	31.1 ± 9.5
Indomethacin	2.5 ± 1.2**	1.0 ± 0.4	1.4 ± 0.3*
Purified enzymes	COX-1 PGE	COX-2 PGE	
Nimesulide	n.i.	90.3 ± 23.6	
Indomethacin	0.6 ± 0.1	4.1 ± 1.3*	

Results are the mean ± SEM (µmol/l), n = 6. In gastric mucosa: n = 6; in the enzyme assays: n = 4, n.i.: no inhibition. * p < 0.05, ** p < 0.01, comparing nimesulide with indomethacin. In stimulated leucocytes, the IC₅₀ for each drug prostanoid could not be calculated since the lowest concentration of the drugs used gave greater than 50 % inhibition.

In gastric mucosa, PGE is thought to be formed predominantly by COX-1, but any infiltrating activated leucocytes could form PGE via COX-2, which might explain the reduction by nimesulide of PGE formation in the gastric mucosal pieces. Alternatively nimesulide might have a different specificity for human gastric mucosal COX-1.

Nimesulide was less potent than indomethacin as an inhibitor of prostaglandin synthesis by human gastric mucosa. Furthermore, in the enzyme-based assay nimesulide did not inhibit COX-1 but was specific for COX-2. These findings are consistent with the effective analgesic/anti-inflammatory activity of nimesulide coupled with better gastric tolerance compared to indomethacin. Moreover, nimesulide has been reported to exert its anti-inflammatory effects partly by other mechanisms [8].

5. References

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