

Effect of Nimesulide Action Time Dependence on Selectivity Towards Prostaglandin G/H Synthase/Cyclooxygenase Activity

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Summary

PGHS (cyclooxygenase, prostaglandin endoperoxide synthase, 8.11.14-icosatrienoate hydrogen donor oxygen oxidoreductase, EC 1.14.99.1) is a bifunctional, membrane-bound hemoprotein that catalyzes both the bisoxygenation of arachidonic acid to form PGG₂ and the peroxidative reduction of PGG₂ to form PGH₂. Recently two forms of cyclooxygenase have been isolated, one (COX-1) being "constitutive", the other (COX-2) being mitogen-inducible. Nimesulide (CAS 51803-78-2) has been shown to inhibit with high selectivity COX-2 without affecting COX-1 activity, so explaining the previous observations about the selectivity of the anti-prostaglandin effect of the drug. The potency of the effect, however, seems to be different according to these studies. The time dependence of COX-2 inhibitors might afford some clues for a better understanding of the mechanism of COX-2 selective inhibition, of the discrepancy between some authors about the potency of the drug and of the relationship between COX-2 inhibition and inhibition of superoxide anion production, an event also characterized by time dependence. So we evaluated the time dependency of the effect of nimesulide on COX-1 and COX-2. COX-1 was isolated from ram seminal vesicles, and COX-2 was from sheep placenta.

Nimesulide inhibited COX-2 activity in a concentration-dependent manner. The inhibition of COX-2 was characterized by time dependence, so the IC₅₀ varied according to the time of pre-incubation (from 70 ± 35 µmol/l to 0.07 ± 0.05 µmol/l). Nimesulide did not affect COX-1 activity until 1 µmol/l and with an IC₅₀ > 100 µmol/l. In conclusion nimesulide's selective inhibitory effect on COX-2 is time-dependent whereas its weak effect on COX-1 is not time-dependent. This observation agrees with the time dependence effect of COX-2 reported by other workers with NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methane sulphonamide) and with flosulide and explains the different values of IC₅₀ reported by other authors. Nimesulide shares with other sulfanilide-like drugs the time dependence of its selective effect on COX-2.

Zusammenfassung

Einfluß der Zeitabhängigkeit der Wirkung von Nimesulid auf die Selektivität gegenüber der Aktivität von Prostaglandin-G/H-Synthase/Cyclooxygenase

PGHS (Cyclooxygenase, Prostaglandin-Endoperoxid-synthase, 8.11.14-Icosatrienoathydrogendonor-Oxygenoxidoreduktase) stellt ein bifunktionelles, membran-gebundenes Hämoprotein dar, das sowohl die Bisoxxygenation von Arachidonsäure zur Bildung von PGG₂ als auch die peroxidative Reduktion von PGG₂ zur Bildung von PGH₂ katalysiert. Vor kurzem wurden zwei Formen von Cyclooxygenase isoliert; die eine (COX-1) ist „konstitutiv“, die andere (COX-2) ist Mitogen-induzierbar. Es wurde nachgewiesen, daß Nimesulid (CAS 51803-78-2) COX-2 mit hoher Selektivität hemmt, ohne die COX-1-Aktivität zu beeinflussen; dies erklärt frühere Beobachtungen über die Selektivität der Prostaglandin-hemmenden Wirkung der Substanz. Diese Studien ergaben jedoch eine unterschiedlich hohe Wirksamkeit. Die Zeitabhängigkeit der COX-2-Hemmer könnte Hinweise liefern zu einem besseren Verständnis des Mechanismus der selektiven COX-2-Inhibition, zur Diskrepanz zwischen den Aussagen einiger Autoren über die Wirksamkeit der Substanz und zur Relation zwischen COX-2-Inhibition und Hemmung der Superoxid-Anionen-Produktion, die ebenfalls durch Zeitabhängigkeit charakterisiert ist. Untersucht wurde daher die Zeitabhängigkeit der Wirkung von Nimesulid auf COX-1 und COX-2. COX-1 wurde aus Samenbläschen des Schafbocks und COX-2 aus der Schafplazenta isoliert. Nimesulid hemmte die COX-2-Aktivität in Abhängigkeit von der Konzentration. Die COX-2-Inhibition war durch die Zeitabhängigkeit gekennzeichnet, d. h. die IC₅₀ variierte in Abhängigkeit von der Präinkubationszeit (von 70 ± 35 µmol/l bis 0,07 ± 0,05 µmol/l). Nimesulid beeinflusste die COX-1-Aktivität erst ab 1 µmol/l bei IC₅₀ > 100 µmol/l. Zusammenfassend ist festzustellen, daß die Selektivität der Inhibitionswirkung von Nimesulid auf COX-2 zeitabhängig ist, während die schwache Wirkung auf COX-1 keine Zeitabhängigkeit aufweist. Diese Beobachtungen über die Zeitabhängigkeit der Wirkung auf COX-2 entsprechen denen anderer Wissenschaftler, die mit NS-398 (N-2-Cyclohexyloxy-4-nitrophenyl)methansulfonamid gearbeitet haben, und erklären die von ihnen berichteten unterschiedlichen IC₅₀-Werte. Wie andere Sulfanilid-ähnliche Substanzen ist die selektive Wirkung von Nimesulid auf COX-2 zeitabhängig.

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

1. Introduction

PGHS (cyclooxygenase, prostaglandin endoperoxide synthase, 8.11.14-icosatrienoate hydrogen donor oxygen oxidoreductase, EC 1.14.99.1) is a bifunctional, membrane-bound hemoprotein that catalyzes both the bisoxygenation of arachidonic acid to form PGG₂ and the

peroxidative reduction of PGG₂ to form PGH₂ [1]. Recently two forms of cyclooxygenase have been isolated [2], one (COX-1) being "constitutive", the other (COX-2) being mitogen-inducible. COX-1 is present in cells under physiological conditions, whereas COX-2 is induced by some cytokines, mitogen and endotoxin in pathological conditions, such as inflammation [3].

Nimesulide¹⁾ (CAS 51803-78-2) has been shown not to affect prostaglandin synthesis in the bronchial tree [4], where constitutive COX-1 exerts a bronchoprotective role [5], and in gastric mucosa [6, 7], where COX-1 preserves mucosal integrity [8]. By contrast nimesulide deeply affects prostaglandin production in inflammatory exudate [6] and in inflammatory cells [9], where prostaglandin production is COX-2-mediated.

Recently nimesulide has been shown to inhibit with high selectivity COX-2 [9, 10] without affecting COX-1 activity, so explaining the previous observations about the selectivity of the anti-prostaglandin effect of the drug. The potency of the effect, however, seems to be different according to these works. In fact Tavares [9] reports an IC_{50} of about 90 $\mu\text{mol/l}$ towards COX-2, whereas Taniguchi [10] reports an IC_{50} of about 7 $\mu\text{mol/l}$. Interestingly nimesulide affects superoxide production in inflammatory cells only after a short-time (5–10 min) preincubation of polymorphonuclear leukocytes, and these observations stimulated studies about a role of nimesulide on cytosolic traffic of enzymes from/to plasma membranes and on the role of cytoskeleton/cAMP mechanism in plasma membrane embedding of cytosolic enzymes [11]. The question is important since Klein [12, 13] recently has pointed out that flosulide, a cyclopentane derivative of nimesulide, irreversibly bound an amino-terminal part of COX-2 by the formation of a Schiff base between the cyclopentane moiety of flosulide and the N-terminal of COX-2. Nimesulide lacks the cyclopentane moiety, yet it is a selective COX-2 inhibitor. The time dependence of COX-2 inhibitors might afford some clues for a better understanding of the mechanism of COX-2 selective inhibition, of the discrepancy between some authors about the potency of the drug and of the relationship between COX-2 inhibition and inhibition of superoxide anion production, an event also characterized by time dependence. Therefore we decided to evaluate the time dependence effect of nimesulide on its selective effect on COX-2.

2. Materials and methods

2.1. Materials

COX-1 (EC 1.14.99.1, isolated from ram seminal vesicles) and COX-2 (isolated from sheep placenta, purity 70%) were from Cayman Chemical Co. (Ann Arbor, MI, USA). Other Chemicals were from Sigma (St. Louis, MO, USA). Nimesulide was from Helsinn SA (Pambio-Noranco, Lugano, Switzerland) and was dissolved in DMSO (0.1% final concentration).

2.2. Methods

One unit of COX-1/COX-2 enzyme was suspended in 0.5 ml of pH 8.0 Tris-HCl buffer (100 mol/l) containing hematin (1 $\mu\text{mol/l}$) and phenol (2 mol/l) as co-factors. The reaction medium was preincubated with drugs for 2, 5, 10 and 15 min at 37 °C, then 100 mol/l of arachidonate was added and incubated for 2 min at 37 °C. To terminate the reaction and extract the excess of arachidonic acid, 2 ml of n-hexane/ethyl acetate (2:1, v/v) was added to the reaction mixture. The aqueous phase was frozen and the organic solvent phase was discarded. The extraction procedure was repeated twice. The amount of PGE_2 was measured by radioimmunoassay using prostaglandin E_2 ^{125}I -RIA Kits (New England Nuclear, Boston, MA, USA).

3. Results

Nimesulide inhibited COX-2 activity in a concentration dependent manner, the maximal inhibition being

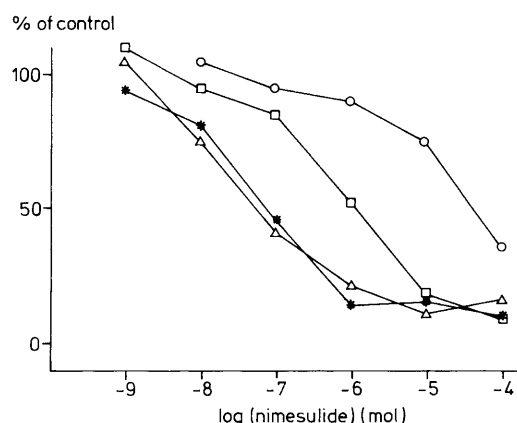


Fig. 1: Effect of preincubation time on inhibition of COX-2. COX-2 was preincubated for 2 (○), 5 (□), 10 (△) and 15 (*) min with nimesulide before the initiation of the reaction with arachidonic acid.

achieved in the 15-min preincubation studies. The inhibition of COX-2 was characterized by the time dependence, so the IC_{50} varied according to the time of preincubation (a representative experiment in Fig. 1). In 2 min pre-incubation studies IC_{50} was $70 \pm 35 \mu\text{mol/l}$ (mean $\pm$ SD of 4 experiments), in 5 min pre-incubation studies it was $3 \pm 2 \mu\text{mol/l}$, in 10 min pre-incubation studies it was $0.05 \pm 0.03 \mu\text{mol/l}$ and in 15 min pre-incubation studies it was $0.07 \pm 0.05 \mu\text{mol/l}$.

Nimesulide did not affect COX-1 activity until 1 $\mu\text{mol/l}$ and with an $IC_{50} > 100 \mu\text{mol/l}$. No time dependence of the effect was observed.

4. Discussion

The main finding of this work is that nimesulide's selective inhibitory effect on COX-2 is time-dependent. This observation agrees with the time dependence effect on COX-2 reported by other workers with NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methane sulphonamide) [14] and with flosulide and its derivatives [13] and explains why Tavares [9] in 5 min pre-incubation studies reported an IC_{50} of about 90 $\mu\text{mol/l}$ and Taniguchi [10] in pre-incubation studies of 10 min an IC_{50} of about 7 $\mu\text{mol/l}$. In this context nimesulide, with other sulfanilide-like drugs, shows the time dependence of its selective effect on COX-2.

Recently the mechanism of action of nimesulide-like drugs has been explored. Ouellet [14] reported that NS-398, a nimesulide-like COX-2 selective inhibitor, is a time-dependent irreversible inhibitor of COX-2, consistent with the formation of an irreversible enzyme-inhibitor complex. Klein [13] reported the formation of an irreversible Schiff base between the cyclopentane moiety of flosulide and some derivatives and an amino group of COX-2. However, nimesulide lacks the cyclopentanone moiety, so the chemical nature of the binding needs further explorations.

There are several chemical classes of inhibitors of COX activity, and at least 12 major chemical series have been reported to affect the production of prostaglandins [15]. The differential sensitivities of COX-1 and COX-2 to NSAIDs is an important issue to establish more potent anti-inflammatory, antipyretic and analgesic effects and to try to obviate to the unwanted side effects (gastrointestinal, bronchial and renal problems).

Drugs that are selective inhibitors of COX-2 have been so far described only in the sulfanilide series. NS-398 (N-(2-cyclohexyloxy-4-nitrophenyl)methane sulphonamide) and SC-58128 (1-(4-fluorophenyl)-3-(trifluoromethyl)-5-(4-methane sulphonylphenyl)-pyrazole) are nimesulide derivatives which share the selective effect on COX-2 with the prototype [16–21].

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

Interestingly nimesulide and its derivatives are also potent inhibitors of superoxide anion release in polymorphonuclear leukocytes [11], and a recent work underlines the link between NADPH-oxidase inhibition and COX-2 expression [22]. Further work is necessary to establish if the COX-2-selective inhibition, NADPH-oxidase inhibition and other cAMP-mediated effects in human polymorphonuclear leukocytes [23, 24] are related phenomena.

5. References

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Protective Effect of Furosemide Combined with Non-steroidal Anti-inflammatory Drugs Administered by Inhalation Route on Guinea-pigs Anaphylaxis Model

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Summary

The exposure of ovalbumin sensitized guinea-pig to an aerosol of the specific antigen causes a respiratory crisis in approximately 100 s (dyspnoea time) associated with a substantial increase in blood concentration of both histamine (from 27.5 ± 1.8 ng/ml to 1570 ± 26 ng/ml; $n = 8$) and thromboxane B_2 (TXB₂, from 0.52 ± 0.03 ng/ml to 18.1 ± 0.6 ng/ml; $n = 8$).

The aerosol treatment of the animals (20 min) with furosemide (CAS 54-31-9, frusemide, FRU), nimesulide (CAS 51803-78-2, NIM), acetylsalicylic acid (CAS 50-78-2, ASA) and indometacin (CAS 53-86-1, INDO) at the concentrations of 1-3-10 and 30 mg/ml,

before ovalbumin challenge, brought about an attenuation of anaphylactic response. The rank order of potency for the prolongation of dyspnoea time was FRU > NIM > ASA > INDO. In these experiments blood evaluation performed at the peak of the dyspnoea time for histamine concentration in the treated animals indicated that whereas FRU ($ED_{25} = 2.14$ mg/ml (1.97–2.38)) and NIM ($ED_{25} = 2.74$ mg/ml (2.37–3.19)) were equiactive in reducing the release of histamine, ASA and INDO were devoid of this activity. On the contrary, the results obtained with ASA and INDO indicated a greater intrinsic activity in antagonizing TXB₂ formation than that shown by the log-dose response curves of NIM and FRU.