

# Antianaphylactic and Antihistaminic Activity of the Non-steroidal Anti-inflammatory Compound Nimesulide in Guinea-pig

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## Summary

Nimesulide (4-nitro-2-phenoxy methane sulfonanilide, Aulin®, Mesulid®) is a non-steroidal anti-inflammatory compound which shows antihistaminic activity and inhibits the immune release of histamine. The antihistaminic activity of this compound is specific for  $H_1$ -receptor and has been demonstrated on isolated strips of guinea-pig trachea and on histamine-induced multiphasic inotropic response in left atria of guinea pig electrically driven. The effect of nimesulide is of non competitive type and, at the concentration of  $1 \times 10^{-5}$  mol/l, is nearly 2 time less potent than pyrilamine (mepyramine) at  $1 \times 10^{-6}$  mol/l. Nimesulide (1.6  $\mu$ mol/kg i.v.) inhibits both bronchoconstriction (69 %) and  $TXB_2$  formation (93 %) induced by histamine (0.05  $\mu$ mol/kg i.v.) in anaesthetized guinea-pigs. In contrast indometacin (1.6  $\mu$ mol/kg i.v.) decreases the generation of  $TXB_2$  (89 %) without affecting the enhancement in tracheal insufflation pressure induced by histamine.

In actively sensitized guinea-pigs both nimesulide and indometacin protect the animals from deadly anaphylactic crisis. The rise in tracheal pressure induced by the antigenic challenge is inhibited of 80 % and 63 % respectively by nimesulide and indometacin (6.4  $\mu$ mol/kg i.v.). At this dose the two compounds reduced of 90 % approximately the immunological release of  $TXB_2$  in the circulation.

The release of histamine, induced by the anaphylactic reaction caused in perfused lungs obtained from actively sensitized guinea-pigs, is lessened by nimesulide ( $EC_{50} = 3.06$ ; fid. lim. 2.59–3.63  $\mu$ mol/l) and potentiated by indometacin ( $EC_{50} = 0.89$ ; fid. lim. 0.67–1.17  $\mu$ mol/l). In these experiments the increase in lung perfusion pressure observed after antigen challenge is linked to  $TXA_2$  and indometacin ( $EC_{50} = 0.62$ ; lim. fid. 0.51–0.74  $\mu$ mol/l) was more potent than nimesulide ( $EC_{50} = 3.35$ ; fid. lim. 2.90–3.88  $\mu$ mol/l) in reducing the generation of this arachidonic acid metabolite.

The capacity of nimesulide to lower the immune release of histamine, and to attenuate the effect of this autacoid at the receptor site of airway smooth muscles, may bear some therapeutical importance in patients with inflammation of the respiratory tract and with a history of allergic airway obstruction.

## Zusammenfassung

Antianaphylaktische und antihistaminische Aktivität des neuen nicht-steroidalen Entzündungshemmers Nimesulid bei Meerschweinchen

Nimesulid (4-Nitro-2-Phenoxy methansulfonanilid, Aulin®, Mesulid®) ist eine nicht steroidale entzündungshemmende Substanz mit antihistaminischer Wirkung, die die immunologische Freisetzung von Histamin hemmt. Die für den  $H_1$ -Rezeptor spezifische antihistaminische Wirkung wurde an isolierten Tracheastreifen von Meerschweinchen und mittels der Histamin-induzierten multiphasischen inotropen Reaktion der elektrisch angetriebenen linken Vorhöfe von Meerschweinchen nachgewiesen. Die Wirkung von Nimesulid ist nicht-kompetitiv und ist bei einer Konzentration von  $1 \times 10^{-5}$  mol/l fast zweimal weniger stark als Pyrilamin (Mepyramin) in einer Konzentration von  $1 \times 10^{-6}$  mol/l. Die durch Histamin (0,05  $\mu$ mol/kg i.v.) induzierte Bildung von  $TXB_2$  (93 %) und Bronchospasmus (69 %) am narkotisierten Meerschweinchen werden von Nimesulid (1,6  $\mu$ mol/kg i.v.) gehemmt. Dagegen vermindert Indometacin (1,6  $\mu$ mol/kg i.v.) die Bildung von  $TXB_2$  (89 %) ohne Beeinträchtigung der durch Histamin induzierten Erhöhung des Trachea-insufflationsdruckes.

Sowohl Nimesulid als auch Indometacin schützen aktiv sensibilisierte Meerschweinchen vor einer tödlichen anaphylaktischen Krise. Der durch die Antigenexposition hervorbrachte Anstieg des Luftröhrendrucks wird zu 80 % bzw. 63 % durch Nimesulid und Indometacin (6,4  $\mu$ mol/kg i.v.) gehemmt. Bei dieser Dosis reduzierten beide Substanzen die immunologische Freisetzung von  $TXB_2$  in die Blutbahn zu etwa 90 %.

Die Freisetzung von Histamin, die durch die in perfundierten Lungen von aktiv sensibilisierten Meerschweinchen hervorbrachte anaphylaktische Reaktion induziert wurde, wird durch Nimesulid ( $EC_{50} = 3,06$ ; Vertr. gr. 2,59–3,63  $\mu$ mol/l) vermindert und durch Indometacin ( $EC_{50} = 0,89$ ; Vertr. gr. 0,67–1,17  $\mu$ mol/l) verstärkt. In diesen Versuchen steht der nach Antigenexposition beobachtete Anstieg im Lungenperfusionsdruck mit  $TXA_2$  in Verbindung. Indometacin ( $EC_{50} = 0,62$ ; Vertr. gr. 0,51–0,74  $\mu$ mol/l) war bei der Verminderung der Bildung dieses Arachidonsäuremetaboliten aktiver als Nimesulid ( $EC_{50} = 3,35$ ; Vertr. gr. 2,90–3,88  $\mu$ mol/l).

Die Fähigkeit von Nimesulid, die immunologische Freisetzung von Histamin zu vermindern und die Wirkung dieses biogenen Amins am Rezeptorort der glatten Muskulatur der Luftwege abzuschwächen, kann für Patienten mit Entzündungen der Atemwege und mit einer Anamnese einer allergischen Verengung der Luftwege therapeutisch von gewisser Bedeutung sein.

**Key words:** Anti-inflammatories, non-steroidal · Aulin® · Histamine, inhibition · Mesulid® · Nimesulide, pharmacology

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## 1. Introduction

Among the methan-sulfonanilide compounds, nimesulide<sup>1)</sup> (4-nitro-2-phenoxy methane sulfonanilide) has been shown to possess a noticeable antiinflammatory, antipyretic and analgesic properties in a variety of pre-clinical pharmacological studies [1–3]. The presence in the chemical structure of this molecule of a functional sulfonanilidic group instead of the more conventional carboxylic and oxydric groups, has been reported to be determinant for both efficacy and safety of nimesulide's mechanism of action in the control of the inflammatory response [4, 5]. This mechanism/s, which in man has not been completely elucidated, seems to involve a tissue selective prostaglandin biosynthesis inhibition [4], and reduction of superoxide-anion production by activated proinflammatory cells [6, 7].

The therapeutical validity of nimesulide has been proved clinically in a large number of patients affected by rheumatic diseases [8–10] and particular emphasis has been given to the results obtained with this drug in the treatment of respiratory-tract inflammatory disorders, pleuritis and chronic bronchopulmonary obstruction included [11–13]. Several chemical mediators of lipidic and non lipidic nature derived from different cellular sources are involved in the inflammatory response and hyperreactivity of the airways [14, 15]. On this ground the use of antiinflammatory drugs has been justified and, when possible, it represents an obligatory intervention in this lung pathology [16, 17]. The above observations and the preliminary results obtained in guinea-pig where, at variance with indometacin, nimesulide antagonized histamine-induced increase in bronchial resistance, prompted us to investigate more precisely the ability of these drugs to interfere with both the release and the effects of histamine in various experimental conditions in normal and actively sensitized guinea-pigs.

## 2. Methods and Material

### 2.1. Anaesthetized guinea-pig experiments

Experiments were performed using male Hartley guinea-pigs ( $400 \pm 45$  g body weight) obtained from BMG-Allevamento (Civitate al Piano, BG, Italy). The animals were housed under conditions of constant temperature and humidity with standard diet (8-GP-17, Charles River, Italy) and water ad libitum. Fasted guinea-pigs were anaesthetized (ethylurethane, 1.3–1.5 g/kg i.p.) and respiration was fully arrested with pancuronium-bromide (2 mg/kg i.v.). Airway insufflation pressure (intratracheal pressure, ITP) and systemic blood pressure (BP) via femoral artery were recorded following the method of Konzett-Rössler [18] using Hewlett-Packard instruments (Hewlett-Packard Co., Waltham, MA, USA). As already described [19], guinea-pigs were prepared for extracorporeal circulation and, according to the blood bathed organ technique [20], circulating thromboxane- $A_2$  like material (TXA<sub>2</sub>-lm) was monitored. For this purpose a stream of arterial blood derived from left carotid artery was allowed to superfuse an helical strip of rabbit aorta (RbA) treated overnight with a mixture of receptor antagonists and indometacin (1 µg/ml) to avoid unwanted responses and to increase the sensitivity of the bioassay tissue to TXA<sub>2</sub>-lm [21]. The ability of histamine (0.05 µmol/kg i.v.) to increase both ITP and TXA<sub>2</sub>-lm in the circulating blood was investigated in guinea-pigs treated with nimesulide (0.4–0.8–1.6 µmol/kg i.v.) and indometacin (1.6 µmol/kg i.v.) administered 3 min before challenge with histamine.

A group of animals were also sensitized to ovalbumin (100 mg/kg i.p. + 100 mg/kg s.c.), according to the procedure described by Piper and Vane [22]. Three weeks later after antigen administration, the animals were anaesthetized and prepared as reported above monitoring BP, ITP and blood TXA<sub>2</sub>-lm. The

anaphylactic response was elicited with ovalbumin (5 mg/kg i.v.) in guinea-pigs treated 3 min before with nimesulide (1.6–3.2–6.4 µmol/kg i.v.) and indometacin (6.4 µmol/kg i.v.). The severity of ITP changes was evaluated according to Davies and Johnston [23] and was expressed as percentage of maximal bronchoconstriction obtained in control animals.

### 2.3. Sensitized perfused guinea-pig lungs

Lungs obtained from ovalbumin-sensitized guinea-pigs were perfused through the pulmonary artery with Krebs-Henseleit solution (37 °C) at the flow rate of 10 ml/min as previously described [24]. In order to monitor histamine and TXA<sub>2</sub>-lm release during the anaphylactic shock (ovalbumin, 1 mg bolus injection), the pulmonary outflow superfused a bank of tissues including a terminal segment of guinea-pig ileum (GPI) and RbA set up in cascade. These tissues were treated with a mixture of receptor antagonists as reported above providing that GPI-superfusing medium was lacking of H<sub>1</sub>-receptor antagonist pyrilamine. Pulmonary perfusion pressure (PP) was also monitored. In order to evaluate histamine and TXA<sub>2</sub> release in the perfusate, pulmonary outflow was collected for 5 min. Histamine was bioassayed according to Levi et al. [25] and Berti et al. [26] in GPI preparation, whereas TXA<sub>2</sub> was determined by specific radioimmunoassay of the stable metabolite thromboxane-B<sub>2</sub> (TXB<sub>2</sub>) [27]. These experiments were performed in groups of lungs pretreated with nimesulide ( $1 \times 10^{-6}$ ,  $3 \times 10^{-6}$  and  $1 \times 10^{-5}$  mol/l) and indometacin ( $3 \times 10^{-7}$ ,  $1 \times 10^{-6}$  and  $3 \times 10^{-5}$  mol/l). Pretreatment started 15 min before antigen and lasted throughout the experiment (10 min).

### 2.4. H<sub>1</sub>-receptor antagonistic activity in vitro

The antihistaminic activity of nimesulide was compared with that of indometacin in isolated trachea and left-atria preparations of male guinea-pigs (350–400 g).

Helical strips of guinea-pig trachea (GPT) were mounted in 10 ml organ bath containing the Krebs-Henseleit solution (37 °C) gassed with a mixture of 95 % O<sub>2</sub> and 5 % CO<sub>2</sub>. Tissues were equilibrated under 1 g resting tension for 60 min prior to addition of drugs and changes of the tonus were measured with an isometric force-transducer connected to a pen-recorder (Basile, Comerio-Varese, Italy). Concentration-response curves to histamine (from  $1.5 \times 10^{-6}$  to  $4 \times 10^{-4}$  mol/l) and acetylcholine (from  $3 \times 10^{-8}$  to  $2 \times 10^{-6}$  mol/l) were repeated in presence of pyrilamine ( $1 \times 10^{-6}$  mol/l), nimesulide ( $1 \times 10^{-5}$  mol/l) and indometacin ( $1 \times 10^{-5}$  mol/l).

According to Hattory and Kanno [28], left atrium, dissected from guinea-pig heart (GPLA), was prepared in a 10 ml organ bath containing aerated Krebs-Henseleit solution (30 °C) with a final pH of 7.4. The preparations (resting tension 2 g.) were electrically driven by rectangular pulses of 1 ms duration and intensity of twice the diastolic threshold and at 1 Hz in frequency delivered from a Grass S-88 stimulator (Grass Instr., Quincy, MA, USA).

Tension developed in the atrium was measured in isometric condition. Time course of the positive inotropic effect of histamine ( $1 \times 10^{-6}$  mol/l), expressed in percents of the values before the addition of the agonist, was repeated in preparations bathed with pyrilamine ( $1 \times 10^{-6}$  mol/l), nimesulide ( $1 \times 10^{-6}$  and  $1 \times 10^{-5}$  mol/l) and indometacin ( $1 \times 10^{-5}$  mol/l). The interference of the two antiinflammatory compounds with the inotropic effect of norepinephrine ( $1 \times 10^{-6}$  mol/l) was also investigated.

### 2.5. Drugs

The following drugs were used: histamine dihydrochloride, acetylcholine bromide, pyrilamine maleate (mepyramine), indometacin, norepinephrine hydrochloride and ovalbumin grade V (Sigma Chem. Co., St. Louis, MO, USA); pancuronium bromide (N. V. Organon, Oss, The Netherlands); nimesulide (Helsinn SA, Chiasso, Switzerland); [<sup>3</sup>H] TXB<sub>2</sub> Ria Kit (Nek-007; New England Nuclear, Boston, MA, USA).

## 3. Results

### 3.1. Anaesthetized guinea-pig experiments

When histamine (0.05 µmol/kg) was injected intravenously in anaesthetized guinea-pigs artificially ventilated, a prompt increase in ITP with lowering of systemic BP was recorded. The increase in lung resistance (peak

<sup>1)</sup> Aulin®, Mesulid®; manufacturer: Helsinn S.A., Chiasso (Switzerland).

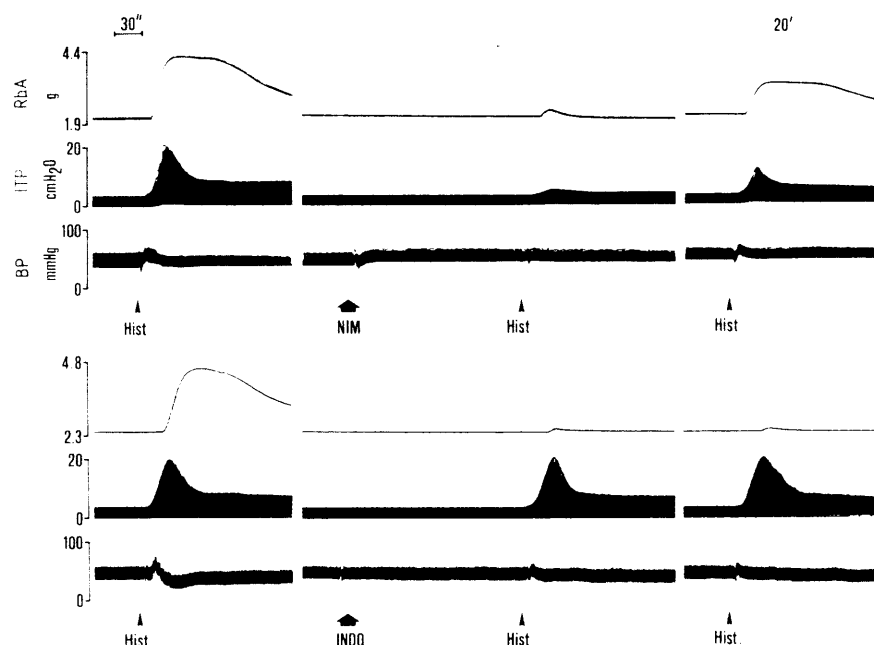


Fig. 1: Effect of nimesulide (NIM, 1.6  $\mu\text{mol/kg}$  i.v.) and indometacin (INDO, 1.6  $\mu\text{mol/kg}$  i.v.) on changes induced by histamine (Hist, 0.05  $\mu\text{mol/kg}$  i.v.) in systemic blood pressure (BP) intratracheal pressure (ITP) and tension of blood bathed rabbit aorta (RbA) in anaesthetized guinea-pig. Drugs were injected 3 min before histamine. In contrast with indometacin, nimesulide markedly reduces the bronchoconstriction caused by histamine.

Table 1: Effect of nimesulide (NIM) and indometacin (INDO) on histamine-induced increase in intratracheal pressure (ITP) and contraction of blood bathed rabbit aorta (RbA) in anaesthetized guinea-pig.

| Treatment<br>( $\mu\text{mol/kg}$ i.v.) | ITP (cm $\text{H}_2\text{O}$ ) |          | RbA (tension in mg) |          |
|-----------------------------------------|--------------------------------|----------|---------------------|----------|
|                                         | M $\pm$ SEM                    | % inhib. | M $\pm$ SEM         | % inhib. |
| Vehicle                                 | 4.25 $\pm$ 0.02                | —        | 15 $\pm$ 2          | —        |
| HIST 0.05 (H)                           | 19.47 $\pm$ 0.82               | —        | 909 $\pm$ 28        | —        |
| NIM 0.4 + H                             | 16.15 $\pm$ 0.54               | 21.8     | 747 $\pm$ 31        | 18.1     |
| 0.8 + H                                 | 12.62 $\pm$ 0.77               | 44.8     | 435 $\pm$ 29        | 53.0     |
| 1.6 + H                                 | 8.96 $\pm$ 0.46                | 69.0     | 80 $\pm$ 13         | 92.7     |
| INDO 1.6 + H                            | 18.45 $\pm$ 1.02               | 6.7      | 117 $\pm$ 8         | 88.6     |

Data (peak effect) are mean values (M)  $\pm$  standard error of the mean (SEM) of 10 animals. The resting tension of blood superfused RbA was 2.12  $\pm$  0.17 g.

Nimesulide

Regr. Funct. for ITP:  $y = 52.8 + 78.5 \log x$  ( $r = 0.996$ );  $\text{ED}_{50} = 0.92$  (0.88–0.97)  $\mu\text{mol/kg}$  i.v.

Regr. Funct. for RbA:  $y = 64.6 + 117.1 \log x$  ( $r = 0.998$ );  $\text{ED}_{50} = 0.75$  (0.73–0.78)  $\mu\text{mol/kg}$  i.v.

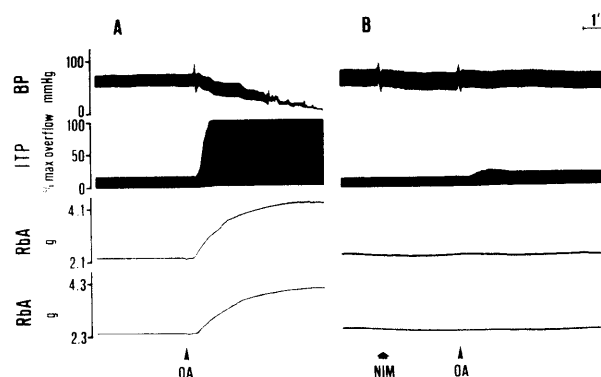


Fig. 2: Nimesulide (NIM) protects sensitized guinea-pigs from anaphylactic crisis. BP, blood pressure; ITP, intratracheal pressure; RbA, blood bathed rabbit aorta; OA, ovalbumin (5 mg/kg i.v.). NIM (6.4  $\mu\text{mol/kg}$  i.v.) was administered 3 min before OA.

effect in 20–30 s) terminated in approximately 90 s and was associated to an increased concentration of  $\text{TXA}_2\text{-Im}$  in the circulation as monitored by the marked contraction of blood superfused RbA (Fig. 1). In animals pretreated with nimesulide the effects of histamine on ITP, BP and RbA-tension were dose-dependently antagonized (Fig. 1 and Table 1). This protective activity of nimesulide was still present 20 min later (Fig. 1).

In clear contrast with nimesulide, indometacin, in spite of its capacity to consistently counteract the effect of histamine on both BP and RbA-contraction did not significantly modify the bronchoconstriction induced by the autacoid, even at the higher dose used (1.6  $\mu\text{mol/kg}$  i.v.). As expected, both nimesulide and indometacin (1.6  $\mu\text{mol/kg}$  i.v.) did not affect the increase in ITP and fall of BP caused by acetylcholine (0.1  $\mu\text{mol/kg}$  i.v.) in this preparation.

When actively sensitized guinea-pigs were challenged with the specific antigen (ovalbumin, 5 mg/kg i.v.) a sustained bronchoconstriction with concomitant progressive fall in BP occurred (Fig. 2). This severe anaphylactic reaction was paralleled by a pronounced contraction of

blood bathed RbA indicating that  $\text{TXA}_2\text{-Im}$  was massively contributing to the immunological reaction (Table 2). All the control animals died in 8–10 min. Nimesulide, given intravenously 3 min before antigen, dose-dependently lowered the increase in both ITP and blood  $\text{TXA}_2\text{-Im}$  (Table 2). The protecting activity of nimesulide was marked at the dose of 6.4  $\mu\text{mol/kg}$  i.v. and was more potent (20 % approximately) than that of indometacin (6.4  $\mu\text{mol/kg}$  i.v.) in preventing the increase in ITP (Table 2). The animals treated with both nimesulide and indometacin were still alive 50 min after antigen administration.

### 3.2. Sensitized-perfused guinea-pig lungs

The addition to the perfusion system of ovalbumin (1 mg) to sensitized lungs brought about a remarkable increase in PP values and concomitant release in the pulmonary outflow of histamine and  $\text{TXA}_2\text{-Im}$  as indicated by the consistent tension developed by GPI and RbA set up in cascade under the lungs. As shown in Fig. 3, the contraction of GPI, maximal in 70–90 s, declined rap-

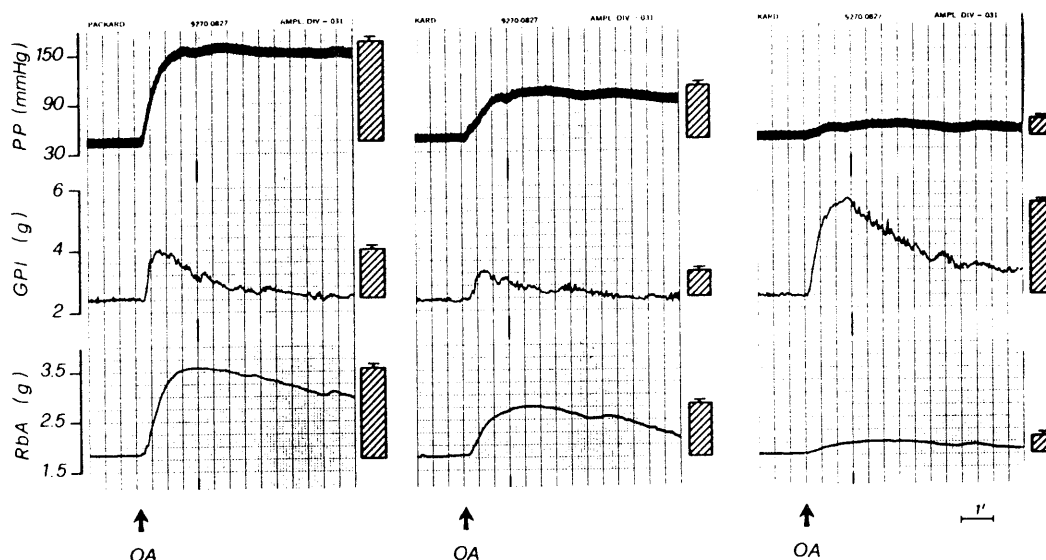


Fig. 3: Changes in perfusion pressure (PP), and resting tension of guinea-pig ileum (GPI) and rabbit-aorta (RbA) during anaphylactic response in sensitized perfused guinea-pig lungs in control (left), nimesulide (middle) ( $3 \times 10^{-6}$  mol/l) and indometacin (right) ( $3 \times 10^{-6}$  mol/l) treated preparations. OA: 1 mg ovalbumin injected through the perfused lungs. Columns represent mean values at the peak effect and vertical bars SEM of 8 experiments. Differences between control, nimesulide and indometacin treated preparations are statistically highly significant ( $p < 0.01$ ). At variance with indometacin, nimesulide significantly reduces the release of histamine.

Table 2: Effect of nimesulide (NIM) and indometacin (INDO) on bronchoconstriction and  $TXA_2$ -Im generation induced by ovalbumin in actively sensitized anaesthetized guinea-pig.

| Treatment<br>( $\mu\text{mol/kg}$ i.v.) | ITP (% max overflow) |          | RbA (tension in mg) |          |
|-----------------------------------------|----------------------|----------|---------------------|----------|
|                                         | M $\pm$ SEM          | % inhib. | M $\pm$ SEM         | % inhib. |
| Vehicle                                 | 0                    | —        | 25 $\pm$ 3          | —        |
| Ovalbumin (OA)                          | 100                  | —        | 2585 $\pm$ 83       | —        |
| NIM 1.6 + OA                            | 76 $\pm$ 1.9         | 24       | 1720 $\pm$ 49       | 33.8     |
| 3.2 + OA                                | 44 $\pm$ 1.7         | 56       | 995 $\pm$ 37        | 62.1     |
| 6.4 + OA                                | 20 $\pm$ 1.4         | 80       | 185 $\pm$ 17        | 93.7     |
| INDO 6.4 + OA                           | 37 $\pm$ 3.0         | 63       | 360 $\pm$ 29        | 86.9     |

Data (peak effect) are mean values (M)  $\pm$  standard error of the mean (SEM) of 8 animals. ITP = intratracheal pressure; RbA = rabbit aorta. The resting tension of blood superfused RbA was  $2.16 \pm 0.15$  g. Drugs were injected 3 min before ovalbumin (5 mg/kg i.v.).

#### Nimesulide

Regr. Funct. for ITP:  $y = 6.3 + 93.0 \log x$  ( $r = 0.990$ );  $ED_{50} = 2.95$  (2.65–3.27)  $\mu\text{mol/kg}$  i.v.

Regr. Funct. for RbA:  $y = 13.0 + 99.4 \log x$  ( $r = 0.993$ );  $ED_{50} = 2.36$  (2.14–2.60)  $\mu\text{mol/kg}$  i.v.

idly in 6–7 min whereas that of RbA reached the peak in 3 min and faded more gradually.

In preparations treated with nimesulide (3  $\mu\text{mol/l}$ ), PP values and tension increase of both GPI and RbA were significantly reduced of 48 %, 52 % and 42 % respectively at the peak effect. In contrast, in preparations treated with indometacin (3  $\mu\text{mol/l}$ ), in spite of a significant reduction of PP values (83 %) and RbA contraction (81 %), the tension developed by GPI increased of 80 % as compared to control preparations. These results indicate that histamine release from guinea-pig lungs during anaphylactic reactions is diminished by nimesulide and increased by indometacin. However, in order to avoid pharmacological interference of relaxant substances on GPI, the total amount of histamine and thromboxane- $B_2$  ( $TXB_2$ ) released in the pulmonary effluent collected for 5 min was determined in groups of lungs perfused before antigen challenge with different concentrations of both nimesulide and indometacin. The results of these experiments are reported in Table 3. These data clearly indicate that, whereas nimesulide caused a concentration-dependent inhibition of the immunological release of his-

Table 3: Effect of nimesulide (NIM) and indometacin (INDO) on histamine and  $TXB_2$  release during the anaphylactic response in ovalbumin-sensitized perfused guinea-pig lungs.

| Treatment<br>( $\mu\text{mol/l}$ ) | Histamine               |        | $TXB_2$        |        |
|------------------------------------|-------------------------|--------|----------------|--------|
|                                    | ( $\mu\text{g/5 min}$ ) | %      | (ng/5 min)     | %      |
| Control                            | n.d.                    | —      | n.d.           | —      |
| Ovalbumin (OA)                     | 25.0 $\pm$ 1.3          | —      | 2691 $\pm$ 99  | —      |
| NIM 1 + OA                         | 19.1 $\pm$ 1.1          | – 23.6 | 2094 $\pm$ 65  | – 22.6 |
| 3 + OA                             | 12.8 $\pm$ 1.7          | – 48.6 | 1441 $\pm$ 89  | – 46.5 |
| 10 + OA                            | 5.3 $\pm$ 0.4           | – 78.9 | 654 $\pm$ 61   | – 75.7 |
| INDO 0.3 + OA                      | 30.5 $\pm$ 1.5          | + 21.9 | 1811 $\pm$ 101 | – 32.7 |
| 1 + OA                             | 38.4 $\pm$ 0.8          | + 53.4 | 973 $\pm$ 57   | – 63.8 |
| 3 + OA                             | 45.3 $\pm$ 2.3          | + 81.1 | 452 $\pm$ 23   | – 83.2 |

Data are mean values  $\pm$  SEM related to 10 perfused guinea-pig lungs. The perfusion with NIM and INDO started 15 min before ovalbumin (bolus of 1 mg) and lasted for 10 min after antigen challenge. n.d. = non detectable.

#### Nimesulide

Regr. Funct. for histamine:  $y = 23.1 \pm 55.3 \log x$  ( $r = 0.97$ );  $EC_{50} = 3.06$  (2.59–3.62)  $\mu\text{mol/l}$

Regr. Funct. for  $TXB_2$ :  $y = 22.1 \pm 53.1 \log x$  ( $r = 0.98$ );  $EC_{50} = 3.35$  (2.90–3.88)  $\mu\text{mol/l}$

#### Indometacin

Regr. Funct. for histamine:  $y = 53.1 \pm 59.1 \log x$  ( $r = 0.95$ );  $EC_{50} = 0.89$  (0.67–1.17)  $\mu\text{mol/l}$

Regr. Funct. for  $TXB_2$ :  $y = 60.7 \pm 50.6 \log x$  ( $r = 0.98$ );  $EC_{50} = 0.62$  (0.51–0.74)  $\mu\text{mol/l}$

tamine, indometacin potentiated the secretion of this autacoid in the pulmonary outflow. The two compounds significantly antagonized the immune formation of  $TXB_2$  from the lung tissues and the higher potency of indometacin ( $EC_{50} = 0.62$   $\mu\text{mol/l}$ , fid. lim. = 0.51–0.74) as compared to that of nimesulide ( $EC_{50} = 3.35$   $\mu\text{mol/l}$ , fid. lim. = 2.90–3.88) on this parameter may reflect a different capacity to inhibit arachidonic acid cyclo-oxygenase enzymes. Furthermore the sustained increase of PP values, observed during the anaphylactic response of the lungs, well correlate with  $TXB_2$  generation, and is therefore linked to arachidonic acid metabolites formed via prostaglandin G/H synthase.

### 3.3. Antihistaminic activity in vitro

In order to evaluate the ability of nimesulide to interfere with histamine  $H_1$ -receptor activation, contractions to

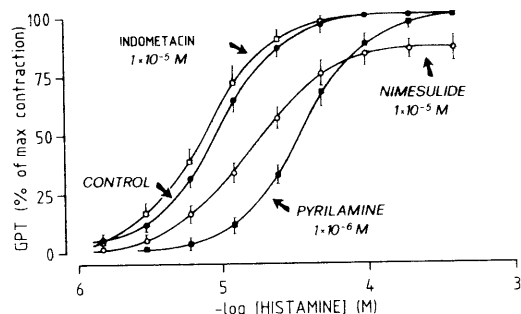


Fig. 4: Concentration-response curves of histamine alone (control) and in presence of nimesulide, indometacin and pyrilamine (mepyramine) on isolated guinea-pig trachea (GPT). Points are means ( $\pm$  SEM) of 5 experiments. Dose ratio (DR) and fiducial limits (95 %) for pyrilamine and indometacin were 3.83 (3.51–4.19), and 0.87 (0.80–0.95), respectively. The DR for nimesulide was not calculated since the parallelism condition from the regression function analysis was not statistically satisfied.

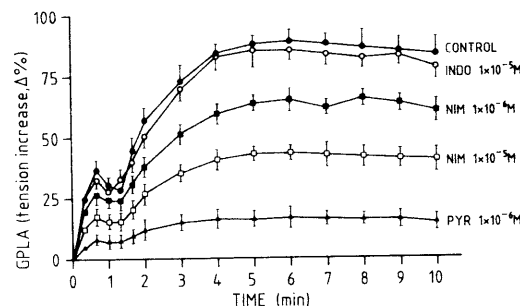


Fig. 5: Time course of the positive inotropic effect produced by histamine ( $1 \times 10^{-6}$  mol/l) in guinea-pig left atria electrically driven in control and in preparations treated with indometacin (INDO), nimesulide (NIM) and pyrilamine (PYR). Ordinates: the developed tension in percent of the values before the addition of histamine. Abscissa: time after addition of histamine. Points are means ( $\pm$  SEM) of 6 experiments. Basal developed tension  $1.84 \pm 0.46$  g. Data were processed according to Dunnett test on AUC (area under the curve) values. Differences between control and nimesulide and pyrilamine treated preparations resulted highly significant ( $p < 0.01$ ).

increasing concentrations of histamine were elicited in helical strips of guinea-pig trachea and left atria preparations.

As shown in Fig. 4, when concentration-response curves for histamine were repeated in presence of nimesulide ( $1 \times 10^{-5}$  mol/l) a significant shift on the right was obtained whereas indometacin, at the same concentration, did not significantly modify histamine-induced contraction of the preparation. The antihistaminic activity of nimesulide appeared to be of non-competitive type since maximal response of the tracheal smooth muscles to the agonist was not achieved in presence of the antagonist (Fig. 4).

The antihistaminic activity of nimesulide on tracheal tissues was rather specific since concentration-response curves for acetylcholine were not modified by this anti-inflammatory agent (data not shown).

The antihistaminic effect of nimesulide was confirmed in multiphasic positive inotropic response to histamine mediated by  $H_1$ -receptors in left atria of guinea-pig. As shown in Fig. 5, when histamine ( $1 \times 10^{-6}$  mol/l) was added to the preparation, a multiphasic inotropic effect was obtained and the increase in force developed was still well maintained after 10 min. This phenomenon was reduced in a concentration-dependent way by nimesulide ( $1 \times 10^{-5}$  mol/l) whereas indometacin was ineffective. As expected, the  $H_1$ -receptor antagonist pyrilamine was markedly more potent than nimesulide in preventing the positive inotropic effect of histamine.

In this preparation the antagonistic activity of nimesulide was specific for histamine  $H_1$ -receptor since the monophasic positive inotropic effect induced by activation of  $\beta$ -adrenergic receptors with norepinephrine ( $1 \times 10^{-6}$  mol/l) was not modified by the anti-inflammatory compound ( $1 \times 10^{-5}$  mol/l). (Data not shown.)

#### 4. Discussion

The data presented here indicate that nimesulide, a compound which has been shown to possess a peculiar anti-inflammatory activity by virtue of a sulfonanilidic group in its chemical structure [4–7] is also endowed with antihistaminic and antiasthmatic properties. The antihistaminic activity of this drug, which is approximately twenty times less potent than pyrilamine, is characterized by a non-competitive mechanism. However, as shown by the in vitro experiments, this effect of nimesulide is rather specific for histamine  $H_1$ -receptor since the stimulating properties of other agonists, such as acetylcho-

line and norepinephrine on the relative preparations, were not affected by this compound. These results also account for the variation of the pharmacological behaviour of nimesulide and indometacin concerning the bronchoconstrictive effect of histamine in anaesthetized guinea-pig. On this occasion in fact, whereas nimesulide antagonized the increase in airway resistance induced by histamine, indometacin was ineffective in spite of its better capacity to impair cyclooxygenase enzymes and  $TXA_2$  generation. On this regard it is well known that the bronchoconstrictive effect of histamine in guinea-pig is primarily due to activation of  $H_1$ -receptor [29] and vagal reflex discharge [30] whereas the generation of  $TXA_2$  induced by histamine does not contribute in a significant extent to changes of airway smooth muscle tonus [19].

Furthermore as shown by the in vitro experiments with perfused lungs, the immune release of  $TXB_2$  seems rather to affect pulmonary circulation since, the reduced biosynthesis of this metabolite caused by both nimesulide and indometacin, is related to the inhibition of perfusion pressure that normally is increased by the anaphylactic response. These data, which are in line with previous in vitro results [4], again demonstrate a different potency between indometacin and nimesulide as prostaglandin inhibitors being the former compound more potent. On this regard, inhibition of neutrophil oxidative metabolism and decreased superoxide anion production has been proposed as an additional mechanism of the anti-inflammatory activity of nimesulide [6].

Another point of interest emerging from the present experiments is the ability of nimesulide to protect guinea-pig from deadly systemic anaphylactic crisis, an acute reaction widely supported by the release of histamine from sensitized mast cells along side with other mediators in target organs such as lungs and heart [13].

The protective mechanism of nimesulide observed in these experiments seems to diverge from that of indometacin when histamine secretion is considered. In fact the release of this autacoid from sensitized perfused lungs of the guinea-pig following specific antigen challenge was significantly reduced in preparations treated with nimesulide and markedly potentiated in lungs perfused with indometacin. This latter observation is consistent with previous results reported by Adcock and coworkers [32] who have analyzed the mechanism of mediator release enhancement after inhibition of cyclooxygenase enzymes in guinea pig lungs. They concluded that this potentiation involves the generation of arachidonic acid metabolites, different from leukotrienes, which facilitate histamine re-

lease. Similar results were also obtained on sensitized human bronchus where cyclooxygenase pathway inhibition resulted in augmentation of antigen-induced bronchial contraction principally due to enhanced release of histamine and other anaphylactic mediators from mast cells [33]. The mechanism whereby nimesulide inhibits the histamine-release reaction remains unclear and the possibility that this compound may have interfered with the early secretory events at the membrane level of sensitized mast cells should be investigated. Whatever the mechanism will be, the findings that nimesulide may control the immunological secretion of histamine along side with attenuation of the effect of this mediator at the receptor site, provide evidence for an unusual pharmacological profile related to a non-steroidal anti-inflammatory agent. This may be of therapeutic value in patients affected by inflammation of the respiratory tract and with a history of allergic airway obstruction. On this regard, Bianco and Robuschi (personal communication) demonstrated that nimesulide does not induced asthma in asthmatic patients who were intolerant to non-steroidal anti-inflammatory drugs.

## 5. References

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