

Inhibition of the Production of Platelet Activating Factor and of Leukotriene B₄ in Activated Neutrophils by Nimesulide Due to an Elevation of Intracellular Cyclic Adenosine Monophosphate

A. T. J. Tool and A. J. Verhoeven

Central Laboratory of the Netherlands Red Cross Blood Transfusion Service and Laboratory for Experimental and Clinical Immunology, University of Amsterdam, Amsterdam (The Netherlands)

Summary

Nimesulide (CAS 51803-78-2) has been shown to exert a marked anti-inflammatory effect in several *in vivo* models of inflammation. Recent studies indicate that nimesulide not only inhibits prostaglandin synthesis in certain cell types, but also has pleiotropic effects on neutrophil functions, including the respiratory burst, integrin-mediated adherence and synthesis of platelet-activating factor (PAF). In the present study, the effect of nimesulide on PAF synthesis was compared with its effect on the production of leukotriene B₄ (LTB₄). Nimesulide dose-dependently inhibited both processes in neutrophils stimulated by serum-treated zymosan (STZ) with a comparable efficacy (IC₅₀ values between 10 and 20 µmol/l). In formyl-methionyl-leucyl-phenylalanine-stimulated neutrophils (treated with cytochalasin B), these IC₅₀ values were 30 and 50 µmol/l for PAF and LTB₄ synthesis, respectively. These results indicate an inhibition by nimesulide of a common step in the release of these lipid mediators, i.e. the activation of phospholipase A₂, possibly by elevating intracellular cAMP. In support of this latter hypothesis, it was observed that nimesulide increased the level of cAMP almost 3-fold after STZ stimulation, whereas in fMLP-stimulated neutrophils these changes in cAMP levels were more dramatic. Furthermore, the inhibitory effects of nimesulide on PAF and LTB₄ production could largely be prevented by addition of H89, an inhibitor of cAMP-dependent protein kinase (PK-A). It is concluded that an increase in intracellular cAMP is instrumental in the observed effects of nimesulide on the release of PAF and LTB₄ by activated neutrophils and that limited availability of arachidonic acid, also the substrate for the cyclooxygenase pathway, may very well contribute to the effects of nimesulide on prostaglandin synthesis observed in other cell types.

Zusammenfassung

Hemmung der Produktion von Plättchen-aktivierendem Faktor und Leukotrien B₄ in aktivierten neutrophilen Leukozyten durch Nimesulid durch Erhöhung von intrazellulärem zyklischen Adenosinmonophosphat

In mehreren *In-vivo*-Entzündungsmodellen zeigte Nimesulid (CAS 51803-78-2) eine deutliche entzündungs-

hemmende Wirkung. Neue Untersuchungen erbringen Hinweise darauf, daß Nimesulid nicht nur bei bestimmten Zelltypen die Prostaglandin-Synthese hemmt, sondern auch pleiotrope Wirkungen auf Funktionen neutrophiler Leukozyten ausübt, wie zum Beispiel auf respiratorischen Burst, Integrin-vermittelte Adhärenz und Synthese des Plättchen-aktivierenden Faktors (PAF). In der vorliegenden Arbeit wurde die Wirkung von Nimesulid auf die PAF-Synthese mit seinen Auswirkungen auf die Produktion von Leukotrien B₄ (LTB₄) verglichen. Nimesulid hemmte beide Vorgänge bei STZ (serum-treated zymosan)-stimulierten neutrophilen Leukozyten dosisabhängig mit vergleichbarer Wirksamkeit (HK₅₀-Werte zwischen 10 und 20 µmol/l). Bei mit fMLP (Formyl-methionyl-leucyl-phenylalanin) stimulierten neutrophilen Leukozyten (mit Cytochalasin B behandelt) betrugen diese HK₅₀-Werte bei der PAF- und der LTB₄-Synthese 30 bzw. 50 µmol/l. Das läßt darauf schließen, daß Nimesulid einen gemeinsamen Schritt der Freisetzung dieser Lipidmediatoren hemmt, z. B. die Aktivierung der Phospholipase A₂, möglicherweise durch Erhöhung des intrazellulären cAMP-Spiegels. Letztere Hypothese wird durch die Beobachtung gestützt, daß Nimesulid den cAMP-Spiegel nach STZ-Stimulierung fast um das Dreifache steigerte, während bei fMLP-stimulierten neutrophilen Leukozyten diese Änderungen des cAMP-Spiegels drastischer waren. Weiterhin konnte die hemmende Wirkung von Nimesulid auf PAF- und LTB₄-Synthese durch Zusatz von H89, einem Hemmstoff der cAMP-abhängigen Proteinkinase (PK-A), weitgehend aufgehoben werden. Daher wird vermutet, daß der Anstieg von intrazellulärem cAMP eine wichtige Rolle bei den beobachteten Wirkungen von Nimesulid auf die Freisetzung von PAF und LTB₄ durch aktivierte neutrophile Leukozyten spielt. Weiterhin scheint die begrenzte Verfügbarkeit von Arachidonsäure, einem Substrat des Cyclooxygenase-Stoffwechselwegs, möglicherweise zu den Wirkungen von Nimesulid auf die Prostaglandin-Synthese, wie sie in anderen Zellarten beobachtet wurden, beizutragen.

Key words: Anti-inflammatories, non-steroidal · Aulin® · CAS 51803-78-2 · Leukotriene B₄ · Mesulid® · Nimed® · Nimesulide · Nisulid® · Platelet-activating factor

1. Introduction

Nimesulide¹⁾ (CAS 51803-78-2, N-(4-nitro-2-phenoxyphenyl)-methanesulfonamide) has been shown to exert a marked anti-inflammatory effect in several *in vivo*

models of inflammation [1, 2]. Initially, inhibition of prostaglandin synthesis [3] has been proposed as explanation for its mechanism of action. Subsequently, inhibition of the neutrophil respiratory burst [4] and scavenging of products derived thereof [5] came into focus. In a previous study [6], we observed that nimesulide interfered with the synthesis of platelet-activating factor (PAF) by activated human neutrophils at concentrations that were without significant effect on the respiratory

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

burst. From this study it could not be concluded at which step the synthesis of PAF was inhibited: at the level of phospholipase A₂ (generating lyso-PAF being the immediate precursor for PAF synthesis) or at the level of acetyl-transferase (converting lyso-PAF into PAF). However, in the same study concomitant inhibition by Nimesulide of activation of the β 2 integrin CD11b/CD18 (instrumental for adherence and migration of neutrophils) was observed, indicating a pleiotropic effect of the drug rather than inhibition of one particular metabolic pathway.

The recent observations of Bevilacqua et al. [7] provide a very good rationale for the pleiotropic effect of nimesulide. These investigators showed an inhibitory effect of nimesulide on the activity of phosphodiesterase type IV present in neutrophil lysates. An inhibition of phosphodiesterase activity by nimesulide in intact cells is likely to result in an increase in the level of cyclic adenosine monophosphate (cAMP), which has been shown in several studies to be of critical importance for neutrophil activation. Increasing intracellular cAMP (e.g. by β -adrenergic stimulation) suppress neutrophil responses including phospholipase A₂ activation [8], whereas lowering of cAMP (induced by adherence) promotes neutrophil activation [9].

The present study was undertaken to further clarify the mechanism by means of which Nimesulide affects PAF synthesis in activated human neutrophils. We compared the effect of nimesulide on PAF synthesis with its effect on the production of leukotriene B₄ (LTB₄). LTB₄, like PAF, is an important chemoattractant for neutrophils [10], also synthesized by activated neutrophils themselves [11] in order to amplify the inflammatory response. The synthesis of LTB₄ requires the release of arachidonic acid from the sn-2 position of phospholipids by the action of phospholipase A₂. If Nimesulide interferes with phospholipase A₂ activation, inhibition of LTB₄ production is likely to occur due to decreased availability of arachidonic acid. We also tested the hypothesis whether an increase in intracellular cAMP is instrumental in the observed effects of nimesulide on the release of these inflammatory mediators by activated neutrophils.

2. Materials and methods

2.1. Materials

Serum-treated zymosan (STZ) was prepared as described [12]. Isobutyl-methylxanthine (IBMX), H89 (N-[2-(p-bromociannamylamino)ethyl]-5-isoquinoline-sulfonamide), cytochalasin B (Cyto B) and formyl-methionyl-leucyl-phenylalanine (fMLP) were obtained from Sigma (St. Louis, MO, USA). Nimesulide was a gift from Helsinn SA (Pambio-Noranco, Lugano, Switzerland). These agents were dissolved in DMSO at 1000 times the final concentration for cell incubations and stored at -70 °C. Incubation medium for the cell incubations contained 132 mmol/l NaCl, 6.0 mmol/l KCl, 1.0 mmol/l CaCl₂, 1.0 mmol/l MgSO₄, 1.2 mmol/l potassium phosphate, 20 mmol/l HEPES, 5.5 mmol/l glucose and 0.5 % (w/v) human serum albumin, pH 7.4.

2.2. Isolation of neutrophils

Blood was obtained from healthy volunteers and was anticoagulated with 0.4 % (w/v) trisodium citrate (pH 7.4). Granulocytes were purified from the buffy coat at 500 ml of blood as described before [13].

2.3. Measurement of platelet-activating factor (PAF)

PAF was measured with a commercially available competitive radioimmuno-assay (New England Nuclear, Boston, MA, USA). Samples were prepared as follows. After activation of the cells (2×10^6 /ml, 0.8 ml) as indicated in the figures, the reaction was stopped by adding 3 ml of methanol/chloroform (2 : 1) with 2 % (v/v) acetic acid added to the methanol. After separation of the phases with 1 ml of chloroform and 1 ml H₂O, the

lower chloroform phase was stored at -70 °C under nitrogen. Subsequently, the chloroform phases were evaporated during centrifugation under vacuum, and resolved in incubation medium. The radioimmunoassay was performed according to manufacturer's instructions. The amount of PAF in the samples were determined from a standard curve, constructed with known amounts of PAF. Recovery of PAF during the whole procedure was more than 90 %.

2.4. Measurement of leukotriene B₄ (LTB₄)

LTB₄ release was measured in the supernatant of activated cells (2×10^6 /ml) with a commercially available enzyme linked immunoassay (Cayman Chemicals Company, Ann Arbor, MI, USA). After activation of the cells as indicated in the figures, cell-free supernatant were prepared by rapid centrifugation for 5 s at $12\,000 \times g$ in an Eppendorf microcentrifuge. After harvesting the supernatants, LTB₄ was determined exactly as recommended by the manufacturers. The amount of LTB₄ in the samples were calculated from a standard curve, constructed with known amounts of LTB₄.

2.5. Measurement of cAMP

Granulocytes (5×10^6 /ml) were stimulated as indicated in the figures in the absence or presence of the phosphodiesterase inhibitor (IBMX (100 μ mol/l) or nimesulide (100 μ mol/l). Reactions were terminated by the addition of 400 μ l cell suspension to 800 μ l of EtOH. After 10 min at room temperature, the mixture was centrifuged in an Eppendorf centrifuge. Supernatant was collected, and the pellet was washed once with 400 μ l of EtOH/H₂O (2 : 1). Both supernatants were pooled, and evaporated at 42 °C during centrifugation under vacuum. Pellet was resuspended in 50 mmol/l Tris with 4 mmol/l EDTA (pH 7.5). Recovery of cAMP during extraction was more than 80 %. cAMP measurement was performed with a commercially available assay system (Amersham International, Amersham, United Kingdom) exactly as recommended by the manufacturer.

3. Results

To determine the production of PAF and LTB₄ by activated neutrophils, purified human neutrophils were stimulated with STZ as a model for phagocytic stimulation. In parallel incubations, the production of PAF (as measured in total extracts) and LTB₄ (as measured in cell supernatants) was measured. Cell supernatants rather than total cell extracts were used for LTB₄ measurements because of less problems with background signals in the assay used (data not shown). However, measurements of LTB₄ release do reflect LTB₄ production by activated neutrophils due to efficient transport of the product to the extracellular space [14]. The neutrophils produced 842 ± 371 pg PAF and 436 ± 24 pg LTB₄ per 10^6 cells ($n = 3$) in the first 10 min following stimulation with STZ particles. In resting neutrophils, these values were below 25 pg/ 10^6 cells. Fig. 1 shows that nimesulide not only inhibited PAF synthesis in these cells, but also dose-dependently inhibited the production of LTB₄. In fact, production of LTB₄ was slightly more sensitive towards nimesulide inhibition than production of PAF: the IC₅₀ amounted to about 10 μ mol/l as compared to about 20 μ mol/l for inhibition of PAF synthesis. It is of further interest to note that the dose-response curve for inhibition of LTB₄ release was rather uniform, whereas the curve for inhibition of PAF synthesis suggested a bimodal effect of the drug (see Discussion).

The effect of nimesulide on lipid mediator release by neutrophils stimulated with (high concentrations of) the chemotactic peptide fMLP was investigated. Under these conditions, neutrophils produced 642 ± 218 pg PAF and 502 ± 177 LTB₄ pg/ 10^6 cells in the first 4 min following stimulation. The presence of nimesulide again caused a dose-dependent inhibition of both processes (Fig. 2), albeit that the IC₅₀ was somewhat higher than observed in STZ-stimulated neutrophils (30 and 50 μ mol/l for PAF and LTB₄ synthesis, respectively). The ability of nimesulide to inhibit not only PAF synthesis

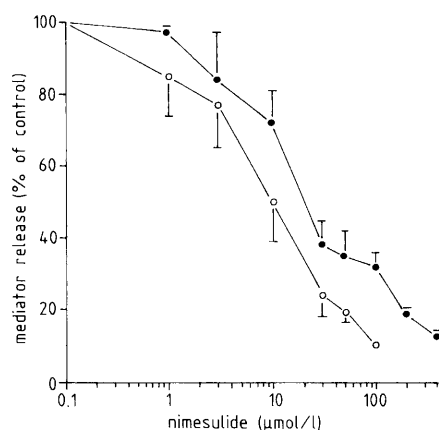


Fig. 1: Effect of nimesulide on PAF synthesis and LTB₄ release in STZ-stimulated human neutrophils. Human neutrophils (2×10^6 /ml) were preincubated for 5 min at 37 °C with increasing concentrations of nimesulide. Control cells received vehicle (DMSO, 0.1 % v/v) only. Subsequently, STZ (1 mg/ml) was added to stimulate the cells. After 10 min of stimulation, incubations were terminated as described in Materials and methods and processed for measurement of PAF (●) and LTB₄ (○) content. Control rates in the absence of nimesulide are given in the text. Results are the mean $\pm$ SEM of 3 different experiments.

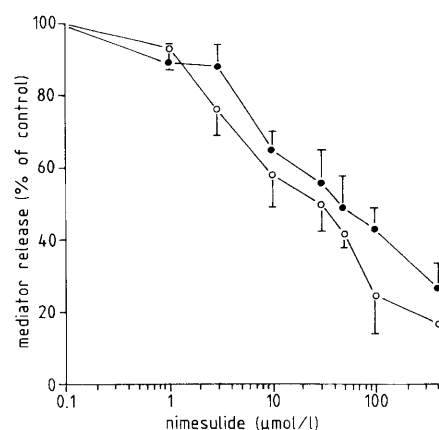


Fig. 2: Effect of nimesulide on PAF synthesis and LTB₄ release in fMLP-stimulated human neutrophils. Human neutrophils (2×10^6) were preincubated for 5 min at 37 °C in the presence of cytochalasin B (5 μg/ml). Cells were then treated with increasing concentrations of nimesulide. Control cells received vehicle (DMSO, 0.1 % v/v) only. After another 5 min of incubation, fMLP (1 μmol/l) was added to stimulate the cells. After 4 min of stimulation, incubations were terminated as described in Materials and methods and processed for measurement of PAF (●) and LTB₄ (○) content. Control rates in the absence of nimesulide are given in the text. Results are the mean $\pm$ SEM of 3 different experiments.

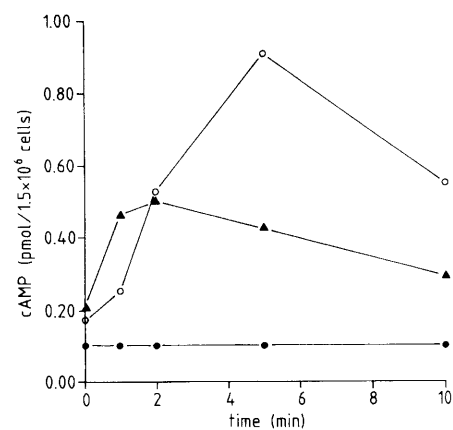


Fig. 3: Effect of nimesulide and IBMX on cAMP content in human neutrophils stimulated with STZ. Human neutrophils (5×10^6 /ml) were preincubated for 5 min at 37 °C in the absence (●) or presence of 100 μmol/l nimesulide (▲) or 100 μmol/l IBMX (○). Control cells received vehicle (DMSO, 0.1 % v/v) only. Subsequently, STZ (1 mg/ml) was added to stimulate the cells. Samples were taken at the time points indicated in the figure and processed for measurement of cAMP content as described in Materials and methods. Values depicted are the mean of 3 different experiments.

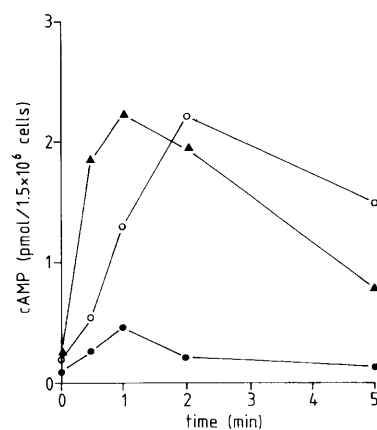


Fig. 4: Effect of nimesulide and IBMX on cAMP content in human neutrophils stimulated with fMLP. Human neutrophils (5×10^6 /ml) were preincubated for 5 min at 37 °C in the presence of cytochalasin B (5 μg/ml). Cells were then treated with 0.1 % (v/v) DMSO (●), 100 μmol/l nimesulide (▲) or 100 μmol/l IBMX (○). After another 5 min of incubation, fMLP (1 μmol/l) was added to stimulate the cells. Samples were taken at the time points indicated in the figure and processed for measurement of cAMP content as described in Materials and methods. Values depicted are the mean of 3 different experiments.

[6], but also (with a similar, although not identical efficacy) the release of LTB₄ strongly suggest an inhibitory effect at a common step in both process, i.e. the activation and/or activity of phospholipase A₂.

To evaluate possible control of phospholipase A₂ activation by changes in cAMP [8], in the next set of experiments the effect of nimesulide on intracellular cAMP levels in neutrophils was investigated. The effect of nimesulide was compared with that of IBMX, a non-specific inhibitor of phosphodiesterases. Preincubation of neutrophils with nimesulide (100 μmol/l) caused a slight increase in cAMP (from 0.10 ± 0.04 to 0.19 ± 0.06 pmol/ 10^6 cells, $n = 6$). Upon stimulation with STZ, the level of cAMP did not change significantly in the absence of phosphodiesterase inhibitors (Fig. 3). However, in the presence of nimesulide the level of cAMP increased almost 3-fold, whereas in the presence of IBMX this increase was even more apparent (Fig. 3). In neutrophils stimulated with fMLP, changes in cAMP levels were more dramatic. Under these conditions, nimesulide was as effective as IBMX in promoting increase in cAMP

(Fig. 4). Taken together, these results support the hypothesis that nimesulide not only inhibits breakdown of cAMP in neutrophil lysates [7], but also in intact cells, thereby affecting an important control mechanism for neutrophil activation.

To further explore the relevance of the effect of nimesulide on cAMP breakdown, it was investigated whether the inhibitory effect of the drug could be reversed by eliminating the activity of cAMP-dependent protein kinase (PK-A). For this purpose, H89, an inhibitor with specificity for PK-A [15], was used. In earlier experiments, Bevilacqua et al. have shown reversal by H89 of the nimesulide inhibition of the respiratory burst induced by fMLP [7]. The results shown in Fig. 5 indicate that, indeed, the presence of H89 (10 μmol/l) reversed the inhibition by nimesulide of the STZ-induced release of LTB₄, albeit that the high production rate in the presence of H89 alone were not reached (Fig. 5, third bar). Similar results were obtained when PAF production was measured as indicator for neutrophil activation (data not shown). In the case of fMLP-stimulated neutrophils, the

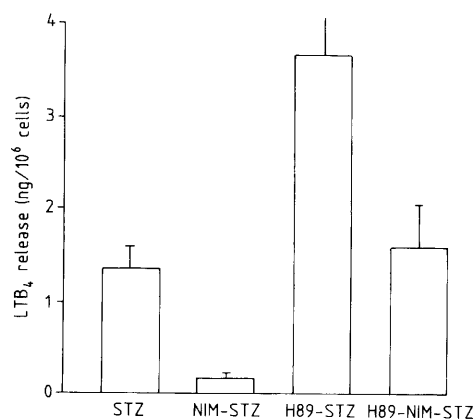


Fig. 5: Effect of H89 on the inhibition by nimesulide (NIM) on STZ-induced LTB₄ release in human neutrophils. Human neutrophils (2×10^6 /ml) were preincubated for 5 min at 37 °C in the absence or presence of inhibitors (H89: 10 μ mol/l; nimesulide: 100 μ mol/l) as indicated in the figure. Control cells received vehicle (DMSO, 0.2% v/v) only. Subsequently, STZ (1 mg/ml) was added to stimulate the cells. After 10 min of stimulation, cell free supernatants were prepared and processed for measurement of LTB₄ content. Results are the mean $\pm$ SEM of 3 different experiments.

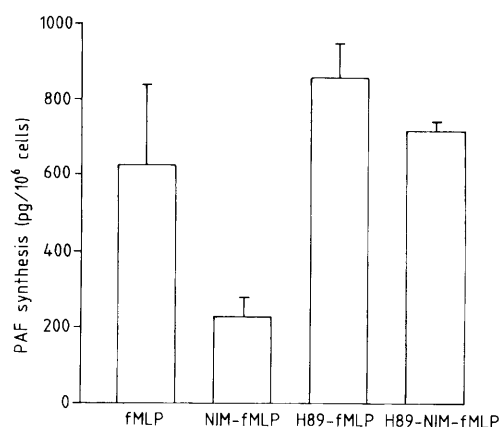


Fig. 6: Effect of H89 on the inhibition by nimesulide (NIM) on fMLP-induced PAF synthesis in human neutrophils. Human neutrophils (2×10^6 /ml) were preincubated for 5 min at 37 °C in the presence of cytochalasin B (5 μ g/ml). Inhibitors (H89: 10 μ mol/l; nimesulide: 100 μ mol/l) were then added as indicated in the figure. Control cells received vehicle (DMSO, 0.2% v/v) only. After another 5 min of incubation, fMLP (1 μ mol/l) was added to stimulate the cells. After 4 min of stimulation, incubations were terminated as described in Materials and methods and processed for measurement of PAF content. Results are the mean $\pm$ SEM of 3 different experiments.

PK-A inhibitor H89 completely prevented the inhibitory effect of nimesulide on PAF synthesis (Fig. 6) or on LTB₄ release (data not shown).

4. Discussion

The results obtained in the present study show that nimesulide not only inhibits PAF synthesis in activated human neutrophils [6] but also the release of LTB₄. This can most readily be explained by an effect of nimesulide on the activation of phospholipase A₂, resulting in a decrease in the production of lyso-PAF (the precursor for PAF) and arachidonic acid, which can be used in the lipoxygenase pathway to produce LTB₄.

It should be noted that the phospholipid precursors for PAF (having an alkyl chain in the sn-1 position) and LTB₄ (having arachidonic acid in the sn-2 position) do not necessarily have to be the same. However, the data obtained in this study suggest, but do not prove, that in activated human neutrophils probably a particular phospholipase A₂ becomes activated acting on a common precursor. Firstly, the neutrophils produced about the

same molar amounts of PAF and LTB₄ upon activation. Secondly, inhibition of PAF and LTB₄ synthesis by nimesulide occurred with a similar, albeit not identical efficacy (Fig. 1). Only at relatively high concentrations of nimesulide, PAF synthesis is somewhat insensitive and higher concentrations of nimesulide are required to achieve complete inhibition. Nevertheless, the data do suggest that one particular phospholipase A₂ is the main target for the nimesulide effect.

Recently, it has become clear that PLA₂ enzymes can be classified in several groups [16]. In man, three prototypes have been sequenced: an intracellular or cytosolic 85-kDa protein (belonging to group IV) and two secretory forms of 14 kDa (group I and II). Cytosolic phospholipase A₂ (cPLA₂) has been shown to prefer arachidonyl-containing phospholipids as substrate and to possess features enabling it to respond to cellular activation signals [17]. Hence, cPLA₂ can be considered as the main candidate to initiate the breakdown of phospholipids leading to the release of arachidonic acid and other lipid-derived precursors, although in certain cell types it has convincingly been shown that this role can also be played by the secretory form (type II) of phospholipase A₂ (sPLA₂) [18, 19]. Recently, the presence of the secretory form (type II) of phospholipase A₂ (sPLA₂) in neutrophils has been reported [20], but results obtained in our laboratory indicate that its expression is much lower than in some other leukocytes (Blom and Verhoeven, unpublished results). Thus, the most likely candidate in human neutrophils to mediate the breakdown of membrane phospholipids resulting in PAF and LTB₄ release is cPLA₂.

Fonteh et al. [8] have reported a negative effect on PLA₂ activation in human neutrophils by agents elevating cAMP. Since Bevilacqua et al. [7] observed that Nimesulide is able to inhibit phosphodiesterase type IV present in neutrophil lysates, it seems reasonable to postulate that nimesulide exerts its inhibitory effect on PAF and LTB₄ production by its effect on cAMP breakdown. In support of this hypothesis, we observed in this study that the (low) level of cAMP in neutrophils prior to stimulation was elevated by nimesulide treatment. Most strikingly, nimesulide greatly enhanced cAMP accumulation after stimulation of the cells (Fig. 3 and 4), thereby strengthening the negative control on PLA₂ activation. In further support for the important role of cAMP in mediating the effect of nimesulide on lipid mediator release, we observed that this effect was largely abolished in the presence of H89, an inhibitor with specificity towards PK-A (Fig. 5 and 6). It has been suggested [21, 22] that the negative control by cAMP on PLA₂ activation is exerted at the level of Raf-1 kinase, thereby preventing the phosphorylation of cPLA₂ (and activation) by MAP kinase [17].

Recent data suggest that nimesulide may act as an inhibitor of the inducible form of cyclooxygenase (COX-2), but not of the constitutive form COX-1 (I. A. Tavares, personal communication). However, our study clearly indicates that nimesulide also effectively inhibits PAF and LTB₄ production in activated human neutrophils and that, most likely, this inhibition is mediated by an inhibitory effect of cAMP on phospholipase A₂ activation. Hence, limited availability of arachidonic acid, also the substrate for the cyclo-oxygenase pathway, may very well contribute to the effects of nimesulide on prostaglandin synthesis observed in other cell types, especially in those cell types expressing phosphodiesterase type IV.

5. References

- [1] Böttcher, I., Schweizer, A., Glatt, M., Werner, H. A., Drugs Exp. Clin. Res. **13**, 237 (1987) – [2] Swingle, K. F., Moore, G. G. I., Drugs Exp. Clin. Res. **10**, 586 (1984) – [3] Vigdahl, R. L., Tukey, R. H., Biochem. Pharmacol. **26**, 307 (1977) – [4] Bevilacqua, M., Vago, T., Beretta, A., Pain Reprod. **31**, 265 (1988) – [5] Ottonello, L., Dapino, P., Pastorino, G., Montag-

nani, G., Gatti, F., Dallegrì, F., *Drugs* **46** (Suppl. 1), 29 (1993) – [6] Verhoeven, A. J., Tool, A. T. J., Kuijpers, T. W., Roos, D., *Drugs* **46** (Suppl. 1), 52 (1993) – [7] Bevilacqua, M., Vago, T., Baldi, G., Renesto, E., Dallegrì, F., Norbiato, G., *Eur. J. Pharmacol.* **268**, 415 (1994) – [8] Fonteh, A. N., Winkler, J. D., Torphy, T. J., Heravi, J., Udem, B. J., Chilton, F. H., *J. Immunol.* **151**, 339 (1993) – [9] Nathan, C. F., Sanchez, E., *J. Cell Biol.* **111**, 2171 (1990) – [10] Palmblad, J., Malmsten, C. L., Uden, A.-M., Radmark, O., Engstedt, L., Samuelsson, B., *Blood* **58**, 658 (1981) – [11] Ford-Hutchinson, A. W., Bray, M. A., Doig, M. V., Shipley, M. E., Smith, M. J. H., *Nature* **286**, 264 (1980) – [12] Goldstein, I. M., Roos, D., Kaplan, H. B., Weissmann, G., *J. Clin. Invest.* **56**, 1155 (1975) – [13] Roos, D., de Boer, M., in: *Methods in Enzymology*, Vol. 132, G. Di Sabato, J. Everse (eds.), p. 225, Academic Press, New York (1986) – [14] Lam, B. K., Gagnon, L., Austen, K. F., Sobrman, R. J., *J. Biol. Chem.* **265**, 13428 (1990) – [15] Chijiwa, T., Mishima, A., Hagiwara, M., Sano, M., Hayashi, K., Inoue, T., Naito, K., Toshioka, T., Hidaka, H., *J. Biol. Chem.* **265**, 5267 (1990) – [16] Mayer, R. J., Marshall, L. A., *FASEB J.* **7**, 339 (1993) – [17] Clark, J. D., Lin, L. L., Kriz, R. W., Ramesha, C. S., Sultzman, L. A., Lin, A.

Y., Milona, N., Knopf, J. L., *Cell* **65**, 1043 (1991) – [18] Murakami, M., Kudo, I., Inoue, K., *J. Biol. Chem.* **268**, 839 (1993) – [19] Balsinde, J., Barbour, S. E., Bianco, I. D., Dennis, E. A., *Proc. Natl. Acad. Sci. USA* **91**, 11060 (1994) – [20] Rosenthal, M. D., Gordon, M. N., Buescher, E. S., Slusser, J. H., Harris, L. K., Franson, R. C., *Biochem. Biophys. Res. Commun.* **208**, 650 (1995) – [21] Vaillancourt, R. R., Gardner, A. M., Johnson, G. L., *Mol. Cell Biol.* **14**, 6522 (1994) – [22] Buhl, A. M., Avdi, N., Worthen, G. S., Johnson, G. L., *Proc. Natl. Acad. Sci. USA* **91**, 9190 (1994)

Acknowledgements

This study was supported by Helsinn Healthcare SA (Pambio-Noranco, Lugano, Switzerland). The authors wish to thank Prof. D. Roos for his continued support.

Correspondence: Dr. A. J. Verhoeven, c/o Publication Secretariat, Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Plesmanlaan 125, 1066 CX Amsterdam (The Netherlands)

Possible Modes of Action of Nimesulide in Controlling Neutrophilic Inflammation

F. Dallegrì^a, L. Ottonello^a, and M. Bevilacqua^b

^aFirst Medical Clinic, Department of Internal Medicine, University of Genova Medical School^a, Genova (Italy), and Department of Endocrinology, L. Sacco Hospital – Vialba^b, Milan (Italy)

Summary

Even in recent years the development of new non-steroidal anti-inflammatory drugs (NSAIDs) appears to be essentially driven by the archaic idea to protect humans from pain and other symptoms of inflammation. Very few attempts have been made to understand if any of these drugs can limit the tissue injury during one or several forms of inflammatory reaction. In studying the events underlying the development of the tissue injury during neutrophilic inflammation, it was found that at least one NSAID, i.e. nimesulide (CAS 51803-78-2), has a high potential to interfere efficiently with the major pathway responsible for neutrophil-dependent histotoxicity. Other chemically related drugs, not classified as NSAIDs but used in disease conditions characterized by neutrophilic inflammation, i.e. dapsone and sulfapyridine, have similar activities. Therefore, it is suggested that derivatives from sulfanilamide (nimesulide, dapsone and sulfapyridine) are the major candidates for developing rational therapeutic anti-inflammatory strategies for controlling the development of tissue injury during neutrophilic inflammation. Also, these molecules might serve to drive the synthesis of new histoprotective anti-inflammatory drugs.

Zusammenfassung

Mögliche Wirkungsmechanismen von Nimesulid bei der Kontrolle neutrophiler Entzündung
Selbst in den letzten Jahren scheint die Entwicklung neuer nicht-steroidaler entzündungshemmender Arznei-

mittel (NSAID) im wesentlichen von dem alten Gedanken, Menschen vor Schmerzen und anderen Symptomen der Entzündung zu schützen, vorangetrieben zu werden. Es wurden nur sehr wenig Versuche unternommen festzustellen, ob irgendeiner dieser Arzneistoffe bei einer oder mehreren Arten entzündlicher Reaktionen Gewebsschädigungen begrenzen könnte. Eine Untersuchung der Vorgänge, die der Gewebsschädigung bei neutrophiler Entzündung zugrundeliegen, zeigte, daß zumindest ein NSAID, nämlich Nimesulid (CAS 51803-78-2) über ein hohes Potential verfügt, auf den wichtigsten, für von neutrophilen Leukozyten abhängige Gewebstoxizität verantwortlichen Stoffwechselweg effizient einzuwirken. Andere chemisch verwandte Arzneistoffe, die nicht zur Gruppe der NSAID gehören, jedoch bei Erkrankungszuständen, die durch neutrophile Entzündung gekennzeichnet sind, verwendet werden, wie z. B. Dapson und Sulfapyridin, weisen eine ähnliche Wirksamkeit auf. Deshalb werden Sulfanilamid-Derivate (Nimesulid, Dapson und Sulfapyridin) als wichtige Anwärter für die Ausarbeitung rationaler, entzündungsbekämpfender Behandlungsstrategien empfohlen, mit denen die Entwicklung von Gewebsschädigungen bei neutrophiler Entzündung unter Kontrolle gehalten werden kann. Diese Moleküle könnten auch dazu dienen, die Synthese neuer gewebsschützender, entzündungshemmender Arzneistoffe voranzutreiben.

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