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Possible Modes of Action of Nimesulide in Controlling Neutrophilic Inflammation

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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. Dapsone und Sulfapyridin, weisen eine ähnliche Wirksamkeit auf. Deshalb werden Sulfanilamid-Derivate (Nimesulid, Dapsone 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®

1. Neutrophilic inflammation: pathophysiological bases for rational anti-inflammatory therapies

Neutrophilic inflammation, i.e. the inflammatory response characterized by the predominant exudation of circulating neutrophils, occurs as a consequence of tissue colonization by invading bacteria or as expression of various non-infectious diseases. The latter diseases include conditions that can be taken as a model: rheumatoid arthritis during acute phases, chronic obstructive pulmonary disease particularly during acute exacerbations, adult respiratory distress syndromes, ulcerative colitis, immune vasculitides and neutrophilic dermatoses such as dermatitis herpetiformis, pyoderma gangrenosum and erythema elevatum diutinum.

Both infectious and non-infectious neutrophilic inflammatory responses are typically characterized by a high risk to develop irreversible tissue injury (Babior 1984, Weiss 1989). Once recruited at tissue sites of inflammation, neutrophils undergo full activation with consequent generation of superoxide (O_2^-) anions (Babior et al. 1984, Winterbourn 1990). In turn, superoxide anions are rapidly dismutated to hydrogen peroxide (H_2O_2). Owing to its low reactivity and lack of charge, H_2O_2 can diffuse easily in inflamed tissue: we can imagine a local pool of H_2O_2 which is rapidly used for generation of more potent oxidants (Test and Weiss 1986). The size of this H_2O_2 pool depends on the number and level of activation of H_2O_2 -generating cells, i.e. locally recruited neutrophils and macrophages (Dallegrì et al. 1987). In spite of the capacity of various cells to degrade H_2O_2 , primarily erythrocytes in transit in the capillary bed (Dallegrì et al. 1986), the majority of the H_2O_2 generated is handled by neutrophil myeloperoxidase (MPO) as a consequence of the extraordinary high affinity of this enzyme for its substrate (Weiss et al. 1982, Test and Weiss 1986, Winterbourn 1986). The final result of these events is the MPO-catalyzed transformation of H_2O_2 into hypochlorous acid (HOCl). HOCl represents the most reactive and toxic oxidant generated during neutrophilic inflammation (Test and Weiss 1986, Weiss 1989). It exerts a variety of unwanted effects, but two of them are particularly dangerous. First, it reacts with tissue cells, inducing depletion of ATP with consequent cell dysfunction and also cell necrosis (Dallegrì et al. 1986, Dallegrì et al. 1988, Weiss 1989, Bernofsky 1991). In part, these activities are mediated by fractions of HOCl which undergo transformation into chloramines as a result of chemical reactions with soluble molecules such as ammonium ions and aminoacids available in the extracellular environment (Grisham et al. 1984, Dallegrì et al. 1986). Second, HOCl inactivates alpha-1-antitrypsin (AT), a glycoprotein able to diffuse easily from the circulation to inflamed tissues (Carp and Janoff 1979, Zaslów et al. 1983, Ossanna et al. 1986, Dallegrì et al. 1992a). AT being the specific inhibitor of neutrophil elastase, its inactivation by HOCl favours the uncontrolled proteolytic activity of elastase itself (Carrell 1986, Crystal 1990, Travis 1988, Janoff 1985, Weiss 1989). The inactivation of AT by HOCl takes place in two ways: a) HOCl directly oxidizes AT molecules by reacting with methionine-358 of AT (Ossanna et al. 1986, Dallegrì et al. 1992a); b) HOCl transforms neutrophil pro-metalloproteases into active enzymes, able to digest AT (Desroschers and Weiss 1988, Michaelis et al. 1990, Ottonello et al. 1994) (Fig. 1). In summary, the bulk of these events can be conceptualized as imbalance between neutrophil-derived oxidants and tissue anti-oxidant systems, tightly linked with an elastase-antielastase imbalance. In other words, neutrophil MPO transforms H_2O_2 into HOCl, responsible for oxidative tissue injuries and AT inactivation which, in turn, results in the elastase-mediated digestion of the intercellular tissue matrix. Other histolytic pathways at sites of neutrophilic inflammation are likely to exist, also as a consequence of the capacity of neutrophil elastase to inactivate certain natural mechanisms devoted to the regulation of pro-in-

flammatory and proteolytic systems (Carrell 1986, Weiss 1989). Nevertheless, at the present status of knowledge, the intervention of these additional pathways remains to be elucidated. Therefore, the histotoxic-histolytic pathways herein presented i.e. HOCl-elastase, coupled by the HOCl-mediated AT-inactivation, appears to be the major determinant of the risk for tissue injury at sites of neutrophilic inflammation rather than a mere over-simplification of events.

2. Ability of nimesulide to restore oxidant/anti-oxidant and elastase/anti-elastase imbalances at sites of inflammation

As synthesized in the previous paragraph, the present knowledge of the physiopathology of neutrophilic inflammatory processes allows the identification of at least a major histotoxic pathway involved in the development of irreversible tissue injuries. This pathway, i.e. the HOCl-generating MPO system responsible for both AT-inactivation and unrestrained elastase activity, appears as an attractive target for therapeutic pharmacologic approaches. A number of NSAIDs inhibit neutrophil function (Halliwell et al. 1988, Abramson and Weissmann 1989). In particular, members of all chemical classes of NSAIDs inhibit neutrophil aggregation (Abramson and Weissmann 1989). Other neutrophil functional responses are affected variably, depending on the individual drug tested (Brooks and Day 1991). Also, some NSAIDs inhibit the binding of various ligands to neutrophil receptors (Abramson and Weissmann 1989). Finally, attention has been focused on two cell responses relevant to the pro-inflammatory and tissue-destructive activity of neutrophils, i.e. the production of oxidants as evaluated by measuring O_2^- generation and the exocytosis of azurophilic granules containing proteolytic enzymes such as elastase. Among different NSAIDs tested, only two drugs have been shown to reduce O_2^- generation after in vivo administration: piroxicam (Kaplan et al. 1984) and nimesulide (Ottonello et al. 1993b). On the other hand, certain NSAIDs can reduce granule enzyme release at high concentrations in vitro, in the presence of the exocytosis-promoting compound cytochalasin B (Neal et al. 1987). Nevertheless, the use of cytochalasin B, generally required to detect the exocytosis of azurophilic granules in neutrophils exposed to soluble agonists, makes the significance of the results questionable. Therefore, with the exception for the above-mentioned data on the O_2^- generation, proofs in favour of NSAID potential to limit neutrophil histotoxicity are substantially lacking.

In the context of a research project on the neutrophil-mediated tissue injury raised by our group some years ago, we tested various drugs. Among the so-called NSAIDs, a compound i.e. nimesulide¹⁾ (CAS 51803-78-2, N-(4-nitro-2-phenoxyphenyl)-methanesulfonamide) was found to efficiently reduce the bioavailability of HOCl in the neutrophil microenvironment and, in turn, to rescue AT from both oxidative and metalloproteolytic inactivation (Dallegrì et al. 1992a, 1992b, Ottonello et al. 1993a). In other terms, this drug has a high potential to reduce the oxidative tissue injury by HOCl and to enhance the development of an extracellular environment rich of active AT, thereby limiting the destructive effects of neutrophil elastase (Ottonello et al. 1993c). As the oxidative HOCl-dependent inactivation of AT is presently claimed as a major determinant of the tissue destruction by elastase (Weiss 1989), its prevention by nimesulide is relevant. Moreover, the drug's ability to reduce the activity of neutrophil metalloenzymes responsible for the proteolytic inactivation of AT should not be underrated (Ottonello et al. 1994). In fact, in comparison with HOCl, the long-lasting ac-

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

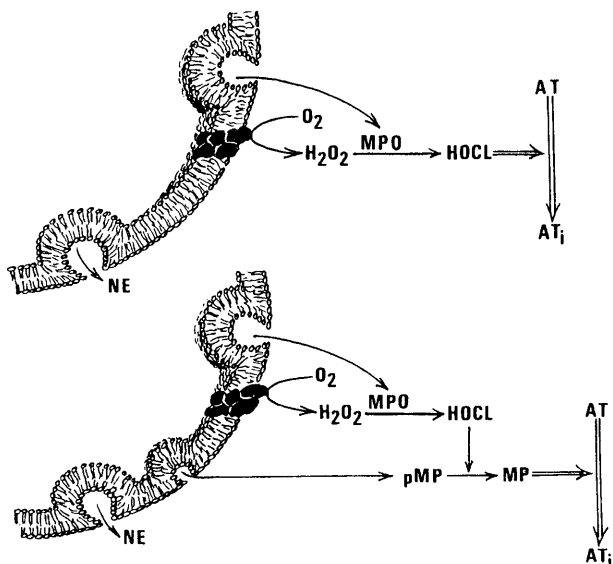


Fig. 1: Inactivation of AT by neutrophils. Neutrophils produce HOCl by the myeloperoxidase (MPO) system. The generated HOCl can directly inactivate AT ($AT \rightarrow AT_i$) oxidatively or it can "switch on" the function of metalloproteases (pMP) which in turn can inactivate AT proteolytically. Owing to AT inactivation, neutrophil elastase (NE) can digest key structural components of the tissue matrix.

tivity of metalloenzymes makes neutrophils capable of inactivating the antiprotease also when the respiratory burst is over. Other drugs, not classified as NSAIDs, behave similarly to nimesulide: dapsone and sulfapyridine (Ottonello et al. 1995) (Fig. 2). Therefore, some comments seem pertinent. First, the drugs proved to be active in reducing HOCl bioavailability and rescuing AT from inactivation (nimesulide, dapsone and sulfapyridine) have structural similarities and are derivatives of sulfanilamide (Gilman et al 1985). Second, these drugs have been reported to have beneficial effects in conditions characterized by neutrophilic inflammation, for example neutrophilic dermatoses and/or soft-tissue neutrophilic inflammatory diseases (Gilman et al. 1985, Moore 1974, Lichtenstein and Fauci 1992). Consequently, on the basis of chemical, pharmacodynamic and clinical criteria, these sulfanilamide-related drugs appear as a well-defined group of anti-inflammatory agents to be considered for future therapeutic strategies to control tissue injury during inflammatory reactions involving neutrophils. It is worthy of note that, other than limiting the digestion of the extracellular matrix, the control of elastase activity by rescued AT can be expected to produce anti-inflammatory effects. In fact, neutrophil elastase promotes microvascular permeability changes leading to plasma exudation (Stroncek et al. 1986), enhances the generation of kinins and the activation of complement by digesting the C1-inhibitor (Carrell 1986), and induces the production of the neutrophil chemotaxin interleukin-8 by certain epithelial cells (Nakamura et al. 1992). Concluding, sulfanilamide derivatives (nimesulide, dapsone, sulfapyridine) appear to have the potential to efficiently interfere with the major pathway leading to histotoxicity during neutrophilic inflammation. Also, these molecules should be taken into consideration for the synthesis of new anti-inflammatory drugs having histoprotective activity, i.e. being potentially capable of modifying the outcome of neutrophilic inflammatory reactions.

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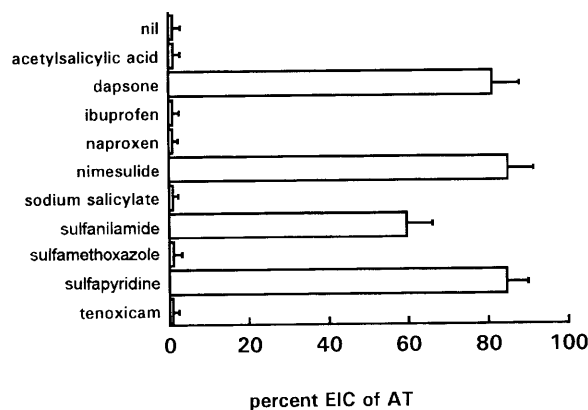


Fig. 2: Drug-mediated rescuing of AT from neutrophil inactivation. Results are expressed as mean $\pm$ 1 SD ($n = 5$). Drug concentration = 50 μ mol/l. Experiments were carried out as previously described (Dallegrì et al. 1992b). Briefly, human neutrophils (2.5×10^6) were stimulated with 10 ng/ml phorbol-myristate-acetate in the presence of 125 μ g purified human AT (final volume 0.25 ml). At the end of the incubation period, the percent elastase inhibitory capacity (EIC) of AT remaining in the supernatant was determined spectrophotometrically.

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