SÃO PAULO MEDICAL JOURNAL

ORIGINAL ARTICLE

Susana Beatriz Veríssimo de Mello, Ieda Maria Magalhães Laurindo, Wilson Cossermelli.

Action of the 4-Nitro-2-Phenoximethanesulphonanilide (Nimesulide) on Neutrophil Chemotaxis and Superoxide Production

Study Carried out at the Laboratório de Investigação em Reumatologia (LIM-17) e Serviço de Reumatologia do Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo.

This research has been partially funded by Fundação para o Desenvolvimento da Reumatologia and by the Conselho dos Fundos Remanescentes da Sociedade Brasileira de Reumatologia.

4-nitro-2-phenoximethanesulphonanilide (nimesulide) is a nonsteroidal anti-inflammatory agent that has been employed in the treatment of inflammatory diseases because of its specific actions on the inflammatory response mechanisms caused by injury. The objectives of this paper were to determine the action of this agent on two notable neutrophil functions, chemotaxis and production of the superoxide anion. These two functions were studied after the neutrophils were pre-incubated with three different concentrations of 4-nitro-2-phenoximethanesulphonanilide (0.1; 0.3 and 0.5 mN). The results obtained herein demonstrated that 4-nitro-2-phenoximethanesulphonanilide-exposed peripheral blood neutrophils from healthy subjects produced significantly less superoxide when challenged by phorbol-mirystate acetate (PMA at 50 ng/ml) or formy-methionil-leucyl-phenilalanine (FMLP 10 -7 M) and opsonizided zymozan (1mg/ml). Additionally, the agent was equally effective in reducing the PMN chemotoaxis when challenged by C5a factor (2% zimozan activated solution), FMLP 10 -9 M and leukotrien (3. 10 -7 M). The results obtained suggest that in addition to its interference in the metabolism of the aracdonic acid, the 4-nitro-2-phenoximethanesulphonanilide may interfere in a more direct fashion with the neutrophil function. This specific action may contribute to its anti-inflammatory activity.

Key words: neutrophil, superoxide, chemotaxis, 4-nitro-2-phenoximethanesulphonanilide.

INTRODUCTION

Polimorphonuclear leukocytes (PMN) are essential elements for the complete inflammatory response. Accumulation of PMN leukocytes at the inflammatory site of injury is characteristic of the acute inflammatory response. During phagocytosis or upon chemical stimulation, PMN leukocytes not only releases several inflammatory substances as lisosomal enzymes and aracdonic acid metabolites, but it also exhibits an increase in oxygen consumption. The latter is recognized as "respiratory burst"(3,4). This increase in oxygen uptake generates the production of superoxide anion (O2-), a free radical formed by a stepwise reduction of the molecular oxygen and its reaction products.

The oxidase enzyme system (nicotinamide, adenine dinucleotide phosphate, NADPH-oxidase), that is responsible by the PNM superoxide generation, is a multicomponent enzyme complex with cytosolic and membrane components. Following cellular activation, this complex becomes coupled to the plasma membrane, which also forms the lining membrane of the phagosoma (7,21,35).

Address for correspondence: *Dr. Susana Beatriz Veríssimo de Mello*
Laboratório de Investigação em Reumatologia
Av. Dr. Arnaldo 455, 3rd floor – CEP 01246-000
São Paulo – SP – Brazil

The superoxide generated by the activated cells is released into the extracellular space and inside the phagocytic vesicles (17). This anion is not believed to be directly involved in bactericidal protection and tissue damage. However, its reactive by-products, namely, hidrogen peroxide, hidroxyl-radicals, hipochlorous acid, N-chloramine and perhaps singlet oxygen, all have an important role in microbicidal activity, inflammation, and in reperfusion injury (5,16,22,33). Whereas superoxide anion is able to inactivate endothelial-derived vascular relaxing factor, its by-products have the ability to reversibly or irreversibly damage compounds of all biochemical classes (22,23,25, 32). These are, nucleic acids, proteins, free amino acids, lipids, lipoproteins, carbohydrates, a large variety of cellular and extracellular macromolecules such as hyluronic acid and collagen (8, 20, 22, 23, 24, 25, 31, 32).

PMN leukocytes can be activated by chemotatic factors, aggregated immnueglobulins and also by non-immune stimuli such as lecithin, ionophores and detergents (35,38). Onceattracted to the inflammatory site, PNM leukocytes can cause irreversible tissue damage by means of releasing proteolytic enzymes and by means of the production of the so-called oxygen-derived active species (OAS). It has been proposed that some non-steroid anti-inflammatory drugs (NSAID), especially 4-nitro-2-phenoximethanesulphonanilide may exert its therapeutic effects independently of suppression of prostaglandin synthesis (28).

These NSAID may act directly on the neutrophils inhibiting aggregation, chemotaxis to the inflammation site, lysosomal enzyme release, OAS production or by annulling the effects of OAS on tissue components (1,26,30). In keeping with this hypothesis, 4-nitro-2-phenoximethanesulphonanilide (nimesulide) administered in a pharmacologically active dose in rats displays an intermediate potency inhibiting prostaglandin synthetase (13,34). Additionally, it does not affect the level of cytoprotective prostaglandins or thromboxane B2 despite its potent anti-inflammatory effect (14,34).

Therefore, the present work was undertaken to determine the influences of 4-nitro-2-phenoximethanesulphonanilide on two different neutrophilic functions, namely, chemotaxis and superoxide production.

METHODS

Chemical reagents

The reagents were obtained from Sigma Chemical Company.

PMA (phorbol-mirystate-acetate), FMLP (formyl-methionil-leucyl-phenylalanine), zymosan, ferrocytochrome C (type VI), leukotriene B4, SOD (superoxide dismutase).

Zymosan was employed in the final concentration of 1mg/ml after an incubation period for 30 minutes with fresh human serum (2:1w/v) at 37°C, washed twice and resuspended in HBSS.

PMA and FMLP were dissolved in DMSO (dimethyl sulfoxide) and subsequently diluted in medium with a resulting concentrations of DMSO (0.1% or less). These concentrations did not produce any detectable effects on PNM viability or any effects of cytochrome C reduction. The final concentrations in the superoxide assay were PMA=50 ng/ml; FMLP = 10^{-7} M, ferrocytochrome C=100μM; superoxide dismutase final concentration –40μm/ml.

In the migratory assay, the stimulant substances were: C5a = 2% zymosan activated plasma; FMLP = 1 0^{-3} M; LTB4 = 3 . 10^{-7} M.

The drug 4-nitro-2-phenoximethanesulphonanilide (nimesulide) was employed in different concentrations near to therapeutic doses.

Isolation of PNM leukocytes

Peripheral blood with heparin was obtained from healthy subjects and PNM leukocytes were isolated by Ficoll-Hypaque density gradient as described by Boyum (11). Mononuclear cells were removed and red cells were lysed twice in the PMN-red cell pellet with a cold isotonic NH4Cl solution. The cells were washed twice with a Hanks balanced salt solution (HBSS) and resuspended with the same buffer solution supplemented with a 10% heat-inactivated fetal calf serum. Subsequently, the cells were counted and cell concentration was adjusted to 10^6 cells/ml. Cellular viability was confirmed by the Tripan blue dye exclusion method.

4-nitro-2-phenoximethanesulphonanilide (nimesulide)

PNM from healthy subjects were incubated with different concentrations of 4-nitro-2-phenoximethanesulphonanilide (0.1; 0.3 and 0.5 mM) for 30 minutes at 37°C before the superoxide production and chemotaxis assays.

Superoxide production assay

After the PMN were incubated with 4-nitro-2-phenoximethanesulphonanilide (nimesulide) superox-

ide (O_2–) production was measured as superoxide dismutase inhibitable reduction of cytochrome C (37). The reaction mixture contained 10^6 non-stimulated and stimulated neutrophils in HBSS and 100µmol of cytochrome C. In order to control 90U samples, SOD was added to determine the level of nonspecific cytochrome C reduction. The mixtures were incubated at 37°C for 20 minutes and the reaction was arrested by placing the tubes in a frozen solution. The absorbance of the supernatanat fluids was determined spectrophotometrically at 550nm.

PMA 50 ng/ml, FMLP (10^{-7} M) or opsonized zymosan (1mg/ml) were used for cell stimulation diluted in HBSS. The amount of produced superoxide was calculated using an extinction coefficient of 21.1 mM-cm (36). The percentage decrease in the amount of O_2-produced in the presence of the drug was calculated relative to control tubes. These tubes demonstrated no spontaneous reduction of ferricytochrome C by a stimulus substance or drugs in the absence of PMN and no significant PMN activity in the absence of stimuli.

Chemotaxis assay

The migratory assay was performed employing a multiwell Boyden chamber as reported elsewhere (29). Aliquots of cell suspension containing 1.5 millions neutrophils were placed in the upper chamber that was separeted from the chemotatic agent in the lower chamber by a 8µm average pore size nitrate filter. The chemotatic agent was substituted by HBSS to measure random migration. Stimuli substances used were FMLP (10^{-9} M), LTB4 ($3 . 10^{-7}$ M) and C5a 25 of zymosan activated plasma). The cells were allowed to migrate in humidified air for 60 minutes at 37°C. Removal of the filters for fixation and staining of the cells followed. Neutrophil migration within the filter was determined under a light microscope employing the "leading front method" (39). The distance from the top of the filter to the farthest point containing two cells was measured under a 40 X magnification light microscope objective.

Duplicate wells were always run for each individual variable. Five fields were counted and averaged for each filter.

Statistical analysis.

Means and standard errors of means (S.E.M.) of all data are presented and compared using the Student's t test or analysis of variance with the significant probability levels of less than 0.05.

RESULTS

SUPEROXIDE PRODUCTION BY NEUTROPHILS

The production of superoxide by PMN leukocytes from healthy subjects was efficiently reduced after the pre-incubation with 4-nitro-2-phenoximethanesulphonanilide (nimesulide) at 0.3 and 0.5 mM. This reduction was more remarkable when induced by PMA (50ng/ml) and it was also observed when FMLP (10^{-7} M) and opsonized zymosan were employed as stimuli (figure 1). The inhibition of superoxide production by 4-nitro-2-phenoximethanesulphonanilide (nimesulide) at 0.1mN was statistically significant only when induced by FMLP.

INFLUENCE OF 4-NITRO-2-PHENOXIMETHANESULPHONANILIDE ON CHEMOTAXIS

In order to investigate the influence of 4-nitro-2-phenoximethanesulphonanilide on chemotaxis, neutrophils were incubated with the drug for 30 minutes at 37°C and immediately resuspended in HBSS with 1% bovine serum albumin for testing. The results depicted in figure 2 indi-

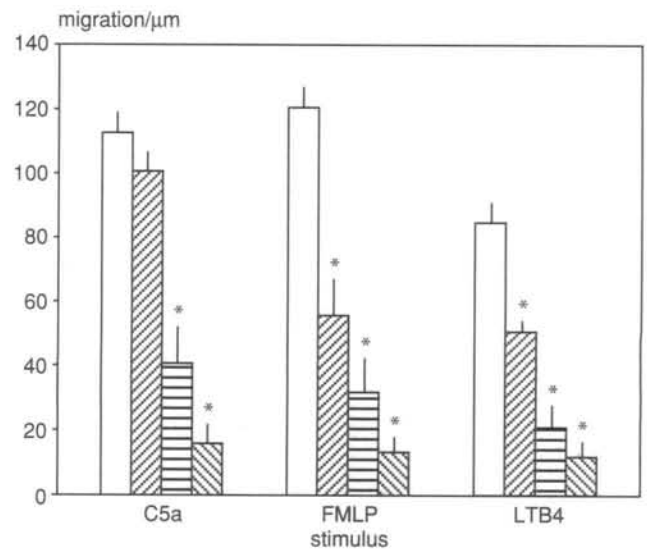

Figure 1 – Superoxide production anion (O2-) by PMN from healthy subjects pre-incubated for 30 minutes at 37°C, with ☐ medium; and with phenoximethanesulphonanilide at ▨ 0.1; ▤ 0.3; ▧ 0.5 mM; PMA at 50 ng/ml; FMLP at 10-7 M and opsonized zymosan at 1mg/ml. These compounds were employed as stimuli. Values are shown as mean +- S.E.M. of the seven individual experiments performed in duplicate.
* < 0.05.

cate that the migratory assay was performed with C5a (25 zymosan activated plasma, FMLP 10-9 M), LTB4 3 .10-7 M). The drug 4-nitro-2-phenoximethanesulphonanilide was inhibitory in all concentrations tested.

DISCUSSION

The results obtained herein demonstrate that 4-nitro-2-phenoximethanesulphonanilide (nimesulide) has a broader but less specific inhibitory action on two PMN functions, namely, chemotaxis and superoxide production. This action proved to occur independently of the type of stimulus employed to activate the neutrophil.

PMN cells were stimulated by different substances with different mechanisms of action.

PMA, opsonized zymosan, FMLP, LTB4, C5a (2% zymosan activated plasma). PMA is believed to directly activate the intracellular protein kinase C bypassing a receptor-mediated signal transduction (19,27). Zymosan stimulates phagocytosis, the complement activation product C5a, LTB4, and the synthetic polypeptide FMLP derived from bacterial endotoxin, all react with distinct and specific sites on the PMN cell membrane (2,6,15,39).

4-nitro-2-phenoximethanesulphonanilide was tested in near therapeutic doses and as in other reports, the authors herein verified no superoxide production activity with 4-nitro-2-phenoximethanesulphonanilide concentrations below 10^{-4} M. The inhibitory drug effect observed on superoxide production and chemotaxis was dose dependent.

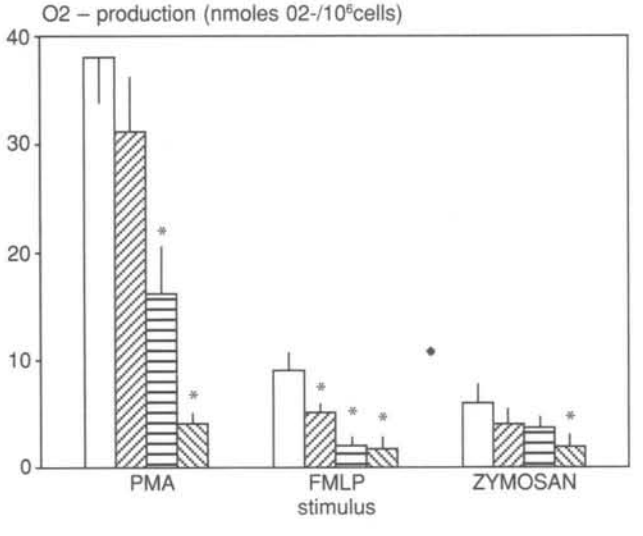

Figure 2 – Chemotatic response of the neutrophils obtained from healthy subjects and pre-incubated with ☐ medium; and with phenoximethanesulphonanilide at ▨ 0.1; ▤ 0.3; ▧ 0.5 mM. Chemoattractants used were C5a (2% zymosan activated serum), FMLP at 10-9 M and LTB4 at 3×10-7 M. Values are shown as mean +- S.E.M. of the seven individual experiments performed in duplicate.
* < 0.05.

The neutrophil responses, chemotaxis and superoxide production both inhibited by 4-nitro-2-phenoximethanesulphonanilide are controlled at different cellular levels of the intracellular signal transduction process. The initial steps of the sequence are shared by both responses (binding of an agonist to its receptor and the interaction of the ligand-receptor complex with a GTP-binding protein). However, the shape change is unaffected by depletion of cytosolic calcium levels and by inhibitors of the protein kinase C while the superoxide production is inhibited under this condition (6,7). It is believed that 4-nitro-2-phenoximethanesulphonanilide interferes with superoxide generation through the decreased protein kinase C translocation from cytosol to neutrophil membranes which has no superoxide scavenger activity (9,10,12). The reduced neutrophil chemotatic activity produced by 4-nitro-2-phenoximethanesulphonanilide may be explained on the same basis. More clear studies are necessary.

The data reported herein support the conclusion of other studies which evaluated the 4-nitro-2-phenoximethanesulphonanilideís inhibitory activity on proteinase release from human leukocytes as well as of chemoluminescence of human leukocytes, rats peritoneal and broncho-alveolar leukocytes. The drug provoked a broad but less specific suppression of these responses, independently of the cell type, its stage of activation and the stimuli employed for leukocyte activation. Previous studies have also demonstrated the 4-nitro-2-phenoximethanesulphonanilide inhibitory action on superoxide production by human neutrophil induced by PMA, FMLP and by zymosan (9,10,12,17).

Our results showed that 4-nitro-2-phenoximethanesulphonanilide produced neutrophil chemotaxis inhibition. Capsoni et al observed no 4-nitro-2-phenoximethanesulphonanilide activity on PMN chemotaxis after cell stimulation performed as in the study herein (zymosan-activated serum and FMLP). However, the different micropore filter size (3μm by 8μm in diameter) and the higher concentration of the chemical compound employed in Capsoniís assays (FMLP at 10^{-8} M and 10% zymosan activated serum against FMLP at 10^{-9} and 2% zymosan activated serum) may account for these conflicting results. Previous assays in our laboratory also failed to demonstrate a 4-nitro-2-phenoximethanesulphonanilide inhibitory activity employing stimulant compounds in high concentrations.

Our data suggest that inhibition of chemotaxis and superoxide production by stimulated neutrophils at the inflammatory site could be an additional anti-inflammatory mechanism by which the 4-nitro-2-phenoximethanesulphonanilide (nimesulide) works.

ACKNOWLEDGMENTS

The authors are grateful to Ms. Maria de F·tima de Almeida for the technical assistance and to Laboratório Asta Médica for supplying the drug and reagents used in this experiment.

REFERENCES

1. ABRAMSON, S.; EDELSON, H.; KAPLAN, H.; LUDEEWIG, R. & WEISSMANN, G. – Inhibition of neutrophil activation by non-steroidal anti-inflammatory drugs. **Am J Med, 74**: 3-6, 1984.
2. ANDERSON, T.; KAHIGREN, C.; LEW, P. D. & STENDAHL, O. – Cell surface expression of fMet-Leu-Phe receptors on human neutrophils. Correlation to cahges in the cytosolic free Ca Level and action of phorbol myristate acetate. **J Clin Invest, 79:** 1226-1233, 1987.
3. BABIOR, B. M. – Owygen-dependent microbial killing by phagocytes. **N Engl Med J, 298:** 659-668, 1978.
4. BABIOR, B. M.; CURNUTTE, J. T. & MCMURRICH, B. J. – The particulate superoxideforming system from human neutrophils. Properties of the system and further evidence supporting its participation in the respiratory burst. **J Clin Invest, 58:** 989-996, 1976.
5. BABIOR, B. M.; KIPNES, R. S. & CURNUTTE, J. T. – Biological defenses mechanism. The production by leukocytes of superoxide, a potential bactericidal agent. **J Clin Invest, 52:** 741-744, 1973.
6. BAGGIOLINI, M. & KERNEN, P. – Neutrophil activation, control of shape change, exocytosis and respiratory burst. **NIPS, 7:** 215-219, 1982.
7. BAGGIOLINI, M.; BOULAY, F.; BADLEY, J. A. & CURNUTTE, J. T. – Activation of neutrophil leukocytes: chemoattractant receptors and respiratory burst. **FASEB, 7:** 1004-1010, 1993.
8. BATES, E. J.; LOWTHER, D. A. & HANDLEY, C. J. – Oxygen free-radicals mediate and inhibition of proteoglycan synthesis in cultured articular cartilage. **Ann Rheum Dis, 43:** 462-469, 1984.
9. BEBILACQUA, M.; VAGO, T. & BERETTA, A. – Nimesulide as an inhibitor of superoxide anion (O2) production by human polymorphonuclear leukocytes. **in** Frontiers in gynecological series – Pain and Reproduction. A. R. GENAZZANI, G. NAPPI, F. FACCHINETTI, E. MARTIGNOMI. ed. The Parthenon publishing Group, New Jersey, USA, 265-272, 1987.
10. BEBILACQUA, M.; NORBIATO, G.; BALDI, G.; BERTORA, P.; VAGO, T. & CHEBAT, E. – Activation of protein kinase C by respiratory burst stimalats desensitizes $beta_2$-adrenoreceptores on human neutrophils. **Drug Invest, 3:** 54-65, 1991.
11. BOYUM, A. – Isolation of mononuclear cells and granuloccytes from human blood. **J Clin Lab Invest, 21:** 77-89, 1968.
12. CAPSONIJ, F.; VENEGONI, E.; MINONZIO, F.; ONGARI, A. M.; MARESCA, V. & ZANUSSI, C. – Inhibition of neutrophil oxidative matabolism by Nimesulide. **Agents Act, 21:** 121-129, 1987.
13. CARR, D. P.; HENN, R.; GREEN, J. R. & BOTTCHER, I. – Comparison of the systemic inhibitor of thromboxane synthesis anti-inflammatory activity and gastro-intestinal toxicity of non-steroidal anti-inflammatory drugs in the rat. **Agents Act, 19:** 374-375, 1986.
14. CESERANI, R.; CASCIARRI, I.; CAVALLETTI, E. & CAZZULANI, P. – Action of Nimesulide on rat gastric prostaglandins and renal function. **Drug Invest, 3:** 14-21, 1991.
15. CHENOWETH, D. E. & HUGLI, T. E. – Demonstration of specific C5a receptor on intact human polymorphonuclear leukocytes. **Proc Natl Acad Sci USA, 75:** 3943-3947, 1978.
16. CROSS, C. E.; HALLIWELL, B.; BORISH., E. T.; PRIOR, W. A.; AMES, B. N.; SAUL, R. L.; MCCORD, J. M. & HARMAN, D. – Oxigen radicals and human disease. **Ann Intern Med, 107:** 526-545, 1987.
17. CURNUTTE, J. T. & BABIOR, B. M. – Biological defense mechanism. The effect of bacteria and serum on superoxide production by granuloccytes. **J Clin Invest, 53:** 1662-1672, 1974.
18. DALLEGRI, F.; OTOONELLO, L.; GATTI, F. & GUIDI, G. – Neutrophil oxidative responses: Cell-directed and scavenging actions of anti-inflammatory drug Nimesulide. **Drug Invest, 3(2):** 71-74, 1991.
19. DE CHATELET, L. R.; SHIRLEY, P. S. & JOHNSTON, JR. R. B. – Effect of phorbol myristate acetate on the oxidative metabolism of human polymorphonuclear leukocytes. **Blood, 47**: 545-554, 1976.
20. DEL MAESTRO, R. T. – An approach to free radicals in medicine and biology. **Acta Physiol Scand, 492:** 153-168, 1980.
21. DEWALD, B.; BAGGLIOLINI, M.; CURNUTTE, J. T. & BABIOR, B. M. – Subcellular localization of the superoxide forming enzyme in human neutrophils. **J Clin Invest, 63:**21-29, 1979.
22. FANTONE, C. J. & WARD, P. A. – Role of wxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. **Am J Pathol, 107(3):** 397-418, 1982.
23. FREEMAN, B. A. & CRAPO, J. D. – Biology of disease – Free radicals and tissue injury. **Lab Invest, 17:** 412-425, 1982.
24. GREEWALD, R. A. & MOY, W. W. – Effect of oxygen-dreived free radicals on hyaluronic acid. **Arthritis Rheum, 23:** 455-459, 1980.
25. LAURINDO, I. M. M.; MELLO, S. B. V. & COSSERMELLI, W. – Participação dos radicais livres de oxigênio na fisiopatologia da artrite reumatóide. **Rev Hosp Clin Fac Med S Paulo, 47:** 38-45, 1992.
26. LEGFELDER, E. – Can anti-inflammatory drugs act as scavengers of oxygen radicals? **Agents Actions, 15:** 56-57, 1984.

27. LEHRER, R. I. & COHEN, L. – Receptor-mediated regulation of superoxide production in human neutrophils stimulated by phorbol myristate acetate. **J Clin Invest, 68:** 1314-1320, 1981.
28. MAGNI, E. – Nimesulide, an overview. **Drug Invest, 3:** 1-3, 1991.
29. MELLO, S. B. V.; FARSKY, S. H. P.; SANNOMYIA, P. & GARCIA-LEME, J. – Inhibition of neutrophil chemotaxis associated with a plasma protein in aging rats: selective depression of cell responses mediated by complement-derived chemo attractants. **J Leuk Biol, 51:** 46-52, 1992.
30. MINTA, J. O. & WILLIAM, M. D. – Some nonsteroidal anti-inflammatory drugs inhibit the generation of superoxide anions by activated polymorphs by blocking ligand-receptor interactions. **J Rheum, 12:** 752-757, 1985.
31. MONBOISSE, J. C.; BRAQUET, P. & BOREL, J. P. – Oxygen-free radical as mediators of collagen breakage. **Agents Actions, 15:** 48-50, 1984.
32. MONCADA, S.; HERMAN, A. G. & VANHOUTTE, P. – Endothelium-derived relaxing factor is identified as nitrio oxide. **TIPS, 8:** 365-371, 1987.
33. SIMPSON, P. J. & LUCCHESI, B. R. – Free radicals and myocardial ischemia and reperfusion injury. **J Lab Clin Med, 110:** 13-25, 1987.
34. SWINGLE, K. F. & MOORE, G. G. I. – Pre-clinical pharmacological studies with Nimesulide. **Drugs Under Exp Clin Res, 10:** 587-597, 1984.
35. TAUBER, A. I. – Protein kinase C and the activation of the human neutrophil NADPH-oxidase. **Blood, 69:** 711-720, 1987.
36. VAN GELDER, B. F. & SLATER, E. C. – The extinction coefficient of cytochrome. **Bloch Biophis Acta, 58:** 593-595, 1962.
37. WEENING, R. S.; WEVER, R. & ROOS, D. – Quantitative aspects of the production of superoxide radicals by phagocytising human granulocytes. **J Lab Clin Med, 85:** 245-252, 1975.
38. WEISS, S. J. & WARD, P. A. – Immune complex induced generations of oxygen metabolites by human neutrophil. **J Immunol, 129:** 309-313, 1982.
39. WILLIAMS, L. T.; SNYDERMAN, R.; PIKE, M. C. & LEKOWITZ, R. L. – Specific receptor sites for chemotactic peptides on human polymorphonuclear leukocytes. **Proc Nat Acad Sol USA, 74:** 1204-1208, 1977.
40. ZIGMOND, S. H. & HIRSH, J. G. – Leukocyte locomotion and chemotaxis: new method for evaluation and demonstration of cell-derived chemotactic factor. **J Exp Med, 137:** 387-410, 1973.
41. WILHELMS, O. H.; LINSSEN, M. J.; LIPPONER, L. & SEILNACHT, W. – Nimesulide, indomethacin. BW 755 C, phenidon, mepacrin and nedocromil inhibit the activation of human and rat leukocytes. **Int J Tiss Reac, XII:** 101-106, 1990.

Resumo

O 4-nitro-2-fenoximetanosulfonanilide (nimesulide), antiinflamatório não esteróide, vem sendo utilizado no tratamento de quadros inflamatórios devido à sua atividade farmacológica nestes mecanismos de resposta as injúrias flogísticas. O objetivo do presente trabalho é estudar a ação deste fármaco sobre duas importantes funções neutrofílicas: quimiotaxia e produção de ânion superóxido após pré incubação com doses crescentes da droga (0,1; 0,3 e 0,5mM). Os resultados obtidos demonstram que a pré-incubação de PMNs coletados do sangue periférico de indivíduos normais com o 4-nitro-2-fenoximetanosulfonanilide reduz significativamente a produção de superóxido por estimulação de PMN com acetato de forbol miristato (PMA 50ng/ml). formil metionil leucil fenilalanina (FMLP 10^{-7}M e Zimozan opsonizado (1mg/ml). Adicionalmente o fármaco se mostrou igualmente efetivo em reduzir a habilidade quimiotáxica de PMNs, frente ao fator C5a (2% de soro ativado por zimozan). FMLP (10^{-9}M) e leucotrieno B4 (3×10^{-7}M). Os resultados obtidos sugerem que além da sua atividade a nível do metabolismo do ácido araquidônico, o 4-nitro-2-fenoximetanosulfonanilide possa interferir diretamente sobre a função neutrofílica, o que provavelmente contribui para sua ação antiinflamatória.