CLINICAL STUDY

Antiasthmatic effects of nedocromil sodium

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Abstract

Accumulating data indicate that bronchial asthma is a chronic inflammatory disease. Airway inflammation and it's control became a principal focus in asthma treatment. Nedocromil sodium is chemically nonsteroidal anti-inflammatory agent for the treatment of mild to moderate asthma. The aim of the study was to determine the effects of NS on bronchial hyperresponsivness and eosinophil activation markers isolated from peripheral blood of asthmatics with mild intermittent asthma. Twenty nine patients of both sexes (17 women, 12 men) with average age of 34 years were recruited into the clinical open study. Bronchial responsivness was assessed by metacholine challenge test prior to starting therapy with NS (preparation Tilade mint aer) and 3rd week and 9th week of follow up. Baseline lung function tests were performed at intervals before treatment and at 3rd and 9th week, respectively. Eosinophil activation markers were determined before and after 3rd and 6th week. Assessement was done by flow cytometry using standard monoclonal antibodies. Bronchial responsivness decreased significantly at 3rd and 9th week of follow up (provocation dose – PD20 increased significantly, $p<0.05$, $p<0.02$, respectively). Improvements of baseline lung function tests were observed in majority of parameters: FVC ($p<0.01$), FEV1 ($p<0.01$), FEV1/FVC ($p<0.01$), MEF 25 ($p<0.03$), MEF 50 ($p<0.01$), MEF 25–75 ($p<0.01$), PEF ($p<0.01$) after 3rd week, however the enhancement of improvement was seen in majority of parameters after 9th week of the study. Significant reduction of eosinophil activation markers expression was noticed: CD69 ($p<0.05$, $p<0.01$) and HLA DR ($p<0.05$, $p<0.05$) after 3rd and 6th week, respectively and CD66 ($p<0.05$) after 3rd week and CD81 ($p<0.05$) after 6th week of follow up.
NS possessed complex antiasthmatic effects resulting in decrease of bronchial responsivness and reduction of eosinophil activation markers in mild asthmatics. The tolerance of the drug was good and no adverse effects have been reported. NS is effective prophylactic drug recommended for use in both adults and children in long-term management of mild asthma. ***(Tab. 2, Fig. 1, Ref. 27.)***
Key words: mild intermittent asthma, bronchial hyperresponsivness, nedocromil sodium, eosinophil activation markers.

Accumulating data indicate that bronchial asthma is a chronic inflammatory disease. In considering new or alternative therapeutic candidates for asthma treatment, those possessing anti-inflammatory properties are of great interest because inflammation is recognized as having central importance in the pathogenesis of asthma.

Nedocromil sodium (NS) is chemically distinct anti-inflammatory agent for the treatment of mild to moderate asthma. Specifically, NS is the disodium salt of pyranoquinoline dicarboxylic acid which represents nonsteroid compound with broad anti-inflammatory effects. The aim of the study was to determine the effect of NS on the bronchial responsiveness, baseline lung function parameters and the expression of eosinophil activation markers in patients with intermittent mild asthma and/or with symptoms of dry cough (an of equivalent bronchial asthma).

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Tab. 1. Parameters of metacholine challenge test and baseline lung functions tests.

	0.week	3rd week	p<	9th week	p*
PD20±SEM (mg)	0.54±0.12	1.11±0.43	0.05	1.48±0.45	0.02
FVC_{exp}±SEM (l)	3.94±0.19	4.13±0.20	0.01	4.06±0.19	NS
FEV1±SEM (l/s)	3.18±0.15	3.42±0.15	0.01	3.43±0.19	0.01
$FEV1/FVC_{exp}$±SEM (%)	81.2±81.53	83.61±1.54	NS	85.7±01.73	0.01
MEF50±SEM (l/s)	3.72±0.26	4.17±0.25	0.01	4.36±0.22	0.01
MEF25±SEM (l/s)	1.77±0.14	1.96±0.17	0.03	2.12±0.15	0.01
MEF25-75±SEM (l/s)	3.74±0.24	4.22±0.24	0.01	4.38±0.21	0.01
PEF±SEM (l^{-1})	7.00±0.42	8.00±0.38	0.01	7.94±0.40	0.01

Notes: p — statistical signigficance of parameters comparing the values before and after 3rd week of therapy, p* — statistical signigficance of parameters comparing the values before and after 9th week of therapy, NS — non significant, SEM — standard mean error, FVC — forced vital capacity, FEV1 — forced expiratory volume in 1st second, Tiffenau index — FEV1/FVC, PEF — expiratory flow, MEF-50 — maximal expiratory flow in 50 % of FVC, MEF — 25- maximal expiratory flow in 25 % of FVC, MEF 25-75 — maximal expiratory flow in. 25-75 of FVC, PD 20 — cumulative dose of metacholine (mg) reflecting 20 % decline (fall) of FEV1

Patients and methods

Twenty-nine asthmatics of both sexes (17 women, 12 men) with average age 34 years (aged from 19 to 57 yrs) were enrolled into the study. They were non smokers, atopics by history and have positive skin test for immediate type of allergy. The patients fulfilled the criteria of intermittent mild asthma and/or they suffered from symptoms of dry cough diagnosed as asthma equivalent (Bosquet, 2000). Prior to starting the study all patients signed the Informed Consent.

Bronchial responsiveness and spirometric parameters

Bronchial responsiveness was determined by using metacholine challenge test (metachacholine produced by company Avocado, Great Britain). Provocation dose 20 (PD20) reflected cumulative dose of metacholine (mg) causing 20 % fall in FEV1 (forced expiratory volume in the 1st second). The following concentration of metacholine solutions were used: 0.06 mg, 0.125 mg, 0.250 mg, 0.5 mg, 1.0 mg, 2.0 mg and 5.0 mg, respectively. Metacholine challenge tests were performed as decribed by Guidelines for metacholine and exercise challenge testing (1999). Patients were informed about the withdrawal of medication that may affect the metacholine challenge test.

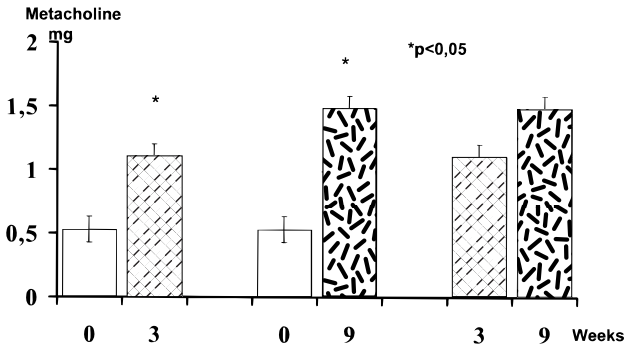

Fig. 1. The effects of NS on bronchial hyperresponsiveness — PD 20 (mg). Notes: PD20 — provocation dose reflecting cumulative dose of metacholine (mg) causing a 20 % fall in FEV1.

Withholdings of medications were generally based on their duration of action (Sterk et al, 1993). In addition, baseline spirometric parameters such as FVC – forced vital capacity, FEV1 – forced expiratory volume in 1st second, Tiffenau index – FEV1/FFVC, PEF – peak expiratory flow, MEF25 – maximal expiratory flow in 25 % of FVC, MEF50, MEF25–75 -50 %, 25–75 % of FVC, respectively. The lung function tests performed in determined intervals (see Study design) using Sanoscop and Provojet (equipment of the company Ganshorn Electronic Medizin, Germany).

Eosinophil activation markers

We used whole – blood immunostaining and flow cytometry based eosinophil selection. The expression of CD16 marker can effectively discriminate between eosinophils and neutrophils. Eosinophil surface molecule expression was effectively studied without the risk of purification-based activation A panel of monoclonal antibodies selected for evaluation of eosinophil activation (anti CD66b, CD81, CD69, HLA DR) produced by Becton Dickinson were selected.

Flow cytometric analysis

The samples were analyzed using a flow cytometer COULTER EPICS XL with an argon laser operating at 488 nm. To analyze the eosinophils within the whole – blood specimen, total granulocytes were gated on unique forward scatter by side scatter characteristics compared with mononuclear cells. Only the cells falling within this total nonmonocytic granulocyte gate were forwarded to the dot plot evaluating TC fluorescein (FITC) or phycoerthrin (PE) and CD16-PE (CD16 FITC) fluorescence intensity. The percentage of eosinophils (CD16-) expressing a specific surface activation marker was deterimend in comparison with the level of FITC (PE) isotype negative control expression. The cut of for negative isotype control staining (vertical/horizontal quadrant marker) was set to be less than 5 % of the extreme tail of the normal distribution for isotype control fluorescence intensity. The experimental samples were recorded as the percentage of eosinophils with FITC (PE) expression beyond this cut off (Mawhorter et al, 1996).

Tab. 2. Eosinophil activation markers.

	0.week	3rd week	p	6th week	p*
CD66±SEM (%)	47.3±4.08	29.7±2.92	0.05	41.1±5.18	NS
CD81±SEM (%)	9.3±1.33	7.8±0.77	NS	6.2±0.72	0.05
CD69±SEM (%)	1.4±0.17	1.2±0.21	0.05	0.82±0.23	0.01
HLADR±SEM (%)	0.5±0.02	0.4±0.17	0.05	0.3±0.16	0.05

Notes: p — statistical significance of parameters comparing the values before and after 3rd week of therapy, p* — statistical significance of parameters comparing the values before and after 6th week of therapy, NS — non significant, SEM — standard mean error

Study design

Prior to the treatment and after 3rd and 9th week of follow up following parameters were measured: forced vital capacity – FVC, forced expiratory volume in 1st second – FEV1, Tiffenau index – FEV1/FFVC, peak expiratory flow – PEF, maximal expiratory flow in 25 % of FVC, 50 %, 25–75 % of FVC, respectively – MEF 25, 50, 25–75.

The bronchial hyperresponsivness was assessed by metacholine challenge test done before and after 3rd and 9th weeks of therapy with NS.

Prior to starting the treatment and after 3rd and 6th week of therapy the eosinophil activation markers from peripheral blood were determined by flow cytometry using standard monoclonal antibodies (anti-CD66b, anti-CD69, anti-CD81, anti-HLDR) see above.

Dose administration and duration of the study

NS (Tilade mint aer, Rhône-Poulenc Rorer, Fisons Ltd., Great Britain) was administered in total daily dose of 8 mg (2x2 puffs daily, each puff contained 2 mg of NS) during 9 weeks. No other medication with bronchodilating or anti-inflammatory effects has been administered concomitantly.

Statistical analysis

The results were statistically evaluated by using Wilcoxon's pair test. The data were analyzed and expressed as the standard mean error. p-Values less than 0.05 were regarded as significant.

Results

Bronchial challenge test and baseline lung function parameters

Bronchial responsivness significantly decreased already after 3rd week of NS administration and significantly enhanced after 9th week of therapy ($p<0.05$, $p<0.02$, respectively) (Tab. 1, Fig. 1). Parameters of lung tests both static and dynamic also changed significantly. FVC increased significantly only after 3rd week of therapy ($p<0.01$), Tiffenau index (FEV1/FVC) changed significantly after 9th week of therapy ($p<0.01$). The effect of increasing FEV1 was noticed after 3rd and 9th week of the study ($p<0.01$, $p<0.01$, respectively) (Tab. 1). Parameters monitoring peripheral lung airways such as MEF 50, 25, 25–75 increased statistically significantly after 3rd week of therapy and enhancement of this phenomenom was observed after 9th week of treatment (Tab. 1).

Parameter of PEF also significantly increased in both controlled intervals ($p<0.01$, $p<0.01$).

Eosinophil activation markers

The expression of eosinophil activation markers CD69 and HLA DR decreased significantly after 3rd and 6th week of therapy (CD69, $p<0.05$, $p<0.01$, HLA-DR, $p<0.05$, $p<0.05$, respectively). The expression of CD66 marker decreased significantly only after 3rd week of therapy ($p<0.05$) and CD81 only after 6th week of therapy ($p<0.05$) (Tab. 2).

Safety and tolerance

NS was well tolerated by patients and no adverse reactions were reported during the study.

Discussion

Bronchial asthma is considered to be a chronic inflammatory disease. Characteristic feature of bronchial asthma is bronchial hyperresponsiveness. In the study we examined patients with mild intermittent asthma and/or with symptoms of dry cough estimated as asthma equivalent. NS nonsteroid anti-inflammatory agent administered at daily dose of 8 mg effectively decreased bronchial responsiveness after 3rd week of therapy and the enhancement of anti-inflammatory effect was demonstrated even after 9th week of follow up. Several authors reviewed benefical effects of NS on bronchial hyperresponsiveness (Rutkowski et al, 1993; Malolepszy et al, 1993). Interesting changes were noticed in lung function tests during the study. No patient has been in evident pulmonary obstruction allowing the performance of challenge tests but despite these findings statistically significant improvement on both static and dynamic lung parameters after therapy were evident. Similar findings have been reported by de Jong et al, who compared the effect of NS with agonist. PEF measured in patients treated with NS was comparable with those treated by ß-agonist In addition NS reduces the diurnal variability of PEF in asthmatic patients (de Jong et al, 1994). Airway responsiveness was lower during treatment with NS compared with regularly inhaled albuterol (Wasserman et al, 1995) The influence on neurovegetative dysbalances seems to be crucial in explanation of mild bronchodilatating effect of NS on large airways (Joos et al, 1989; Verleden et al, 1991) while the effects manifested on peripheral airways are likely to be caused by it's anti-inflammatory properties.

Bronchial asthma represents a chronic inflammatory disease with infiltration of mucosa by eosinophils, mastocytes, T lymphocytes and other cells. Mediators of inflammation are released from activated cells and change the physiology of the airways (Davies et al, 1997). Crucial for this process seems to be the "travelling of cells" from blood stream to mucous tissue in bronchi. The interaction between leukocytes and endothelial cells and epithelial cells mediated through the activation and the expression of adhesion molecules is of importance.

In our study the reduction of the expression of activation markers on peripheral eosinophils was demonstrated. This phenomenon is probably caused by cell membrane stabilizing effects by NS. In this field several authors investigated the influence of NS on the expression of adhesion molecules assessed on epithelial cells, eosinophils and mastocytes located in mucous tissue of bronchi (Barnes et al, 1995; Bleecker et al, 1996; Bleecker et al, 1998; Abdelaziz et al, 1997; Sacco et al, 1999). Reduction of both activation and the expression of adhesion molecules has been demonstrated. NS contribute to inhibition of allergic inflammation by decreasing sIL-2R and sICAM serum levels (Stelmach et al, 2002). Similar effects were seen on PBMC (peripheral blood monocytes) and granulocytes in asthmatics after administration of cromolyn – agent with similar mode of action as NS (Horváthová et al, 1998; Jahnová et al, 1998). Antiasthmatic effects of NS seem to be complex and are determined by the effects on neurovegetative system of lung and anti-inflammatory properties. Novel potential mechanism for the prevention of asthma in relation with NS administration have been reported by Loh et al (1996). These results strongly suggest that NS inhibits IgE isotype switching by inhibition of deletional switch recombination. NS was effective in inhibition of IgE synthesis by human B cells. Inhibition effect of NS on IL-4 serum level and IgE synthesis and on IL-8 release from nasal mucosa of patients with nasal polyps have been also reported (Stelmach et al, 2002; Xaubet et al, 2001).

Inhaled corticoids are the most effective agents for primary control of patients with persistent asthma however these possessed systemic adverse effects when administered in medium doses. Corticoids suppress adrenal function and growth in children (Allen et al, 1998). Withdrawal from prolonged use of inhaled medium-dose corticoids may be associated with clinically significant adrenal insuficiency also in adults (Albert et al, 1998).

Using of NS represents the alternative therapeutic approach to ensure steroid sparing effects (Boulet et al, 1990; Rutkowski et al, 1993; Albert et al, 1998) and to lower the risk of adverse effects of corticoids.

In addition, in mild to moderate asthma there is a comparable efficacy of NS with inhaled corticosteroids and in addition the nonsteroid drugs have higher safety (Konig, 2000).

An increased understanding of the pathogenesis of asthma has led to improved treatment outcome for chronic asthma. Data on clinical efficacy, safety and anti-inflammatory properties support the current position of NS as an effective first line prophylactic drug recommended for use in both adults and children in management of mild asthma and potentialy as steroid sparing agent.

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