

Influence of terbutaline on natural killer cell activity

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Studies on the effect of cAMP-inducing agents on NK activity have been contradictory. The aim of this study was to investigate the acute effects of beta-agonists on NK activity *in vivo* in 15 asthmatics and 3 healthy volunteers. Blood samples of NK activity were taken at regular intervals after placebo and after subcutaneous injection of 7 µg/kg of terbutaline. NK activity was measured by the standard 4-h Chromium⁵¹ release assay against the leukemic line K 562 at a 50:1 effector/target cell ratio. Compared with placebo, terbutaline induced within 30-60 min a significant increase in NK activity which lasted less than 2 h. Further studies are necessary to investigate the effect of long-term beta-agonist treatment on NK activity.

Key words: antiasthmatic drugs; beta-agonists; bronchial asthma; cyclic AMP; natural killer cell activity.

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The results of previous studies on the effect of cAMP-inducing drugs on natural killer (NK) activity have been contradictory. Katz et al. (6) showed that agents which cause a rise in intracellular and extracellular cAMP, for instance isoproterenol, significantly suppressed human NK activity *in vitro*. On the other hand, the stimulation of NK cells by a short exposure to cAMP-elevating drugs has also been reported (9, 10). In view of these findings we decided to study the acute *in vivo* effect of terbutaline used in the treatment of asthma on NK cell activity.

PATIENTS AND METHODS

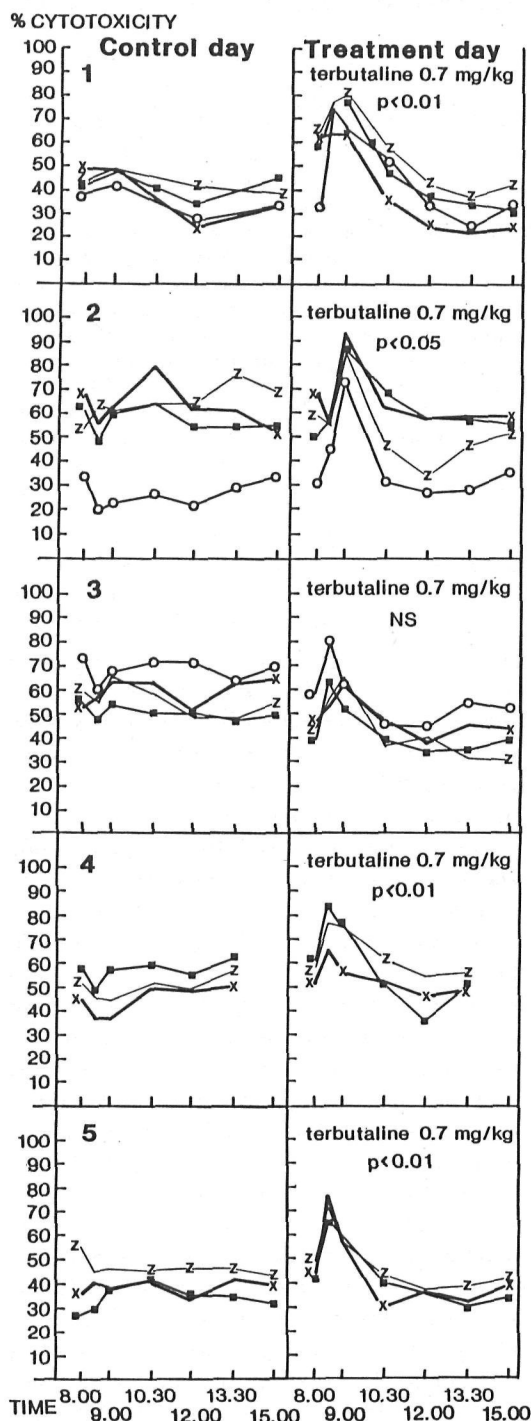
Thirteen men and 2 women, non-smoking, with mild or moderate asthma participated in the study. The mean age was 31 years, range 17-52 years. All patients met the criteria of asthma (1) and showed a variation in the peak expiratory flow of 20% or more spontaneously or a fall in PEF of 20% or more on routine challenge with histamine, antigen, or exercise. Their mean basal FEV₁ was 4.256 l BTPS with an SD of ± 0.87 , this being $91.78 \pm 11.9\%$ of the predicted

values. Twelve patients showed one or more positive reactions when skin prick-tested with a routine panel of allergens. Four patients needed some daily medication for their asthma. All patients suffered from seasonal or perennial rhinitis and 8 from atopic eczema.

Two healthy men aged 46 and 36 and one healthy woman aged 25 participated in experiment 5.

Experiments 1-5 were single blind, in that the physicians (B.S-A., E.V., K.K.) knew which drug was tested, but the patients and the laboratory personnel did not. Informed consent was given by each patient. The experiments were approved by the hospital Ethics Committee and conformed to the Helsinki Declaration of 1975.

Theophylline was discontinued 24 h and beta-agonists 12 h prior to collection of first blood sample, and were not allowed throughout the study period. Topical steroids, antihistamines, and DSCG were stopped 72 h prior to the test. None of the patients used any other medication. The trial was conducted in 5 sessions. Each session included a control day and a



testing day. The patients arrived for clinical assessment on the evening before the control day and stayed in the hospital during the experiment. The healthy volunteers in experiment 5 were tested in the laboratory. Samples for baseline NK activity were taken at the same time of day as the samples on the treatment day. On the treatment day the test drug was given immediately after the first blood sample had been taken. The timing of the blood samples is shown in Fig. 1. The test drug was terbutaline sulphate 7 µg/kg given subcutaneously. During the control day physiological saline was given subcutaneously as placebo.

In the asthmatics peak expiratory flow was measured with a Wright peak flow meter at the time of each blood sampling. Three measurements were used each time and the highest value recorded.

Purification of mononuclear cells, cytotoxic assay, and morphological analysis of mononuclear cells were performed as previously described (12). Briefly, peripheral blood mononuclear cells were isolated from heparinized blood samples by Ficoll-Isopaque density gradient centrifugation and subsequent filtration through nylon wool columns. Natural killer activity was measured by the standard 4-h Chromium⁵¹-release assay against the leukemic cell line K562 at 50:1 effector/target cell ratio.

The statistical two-tail analysis was performed using a paired t-test (12).

RESULTS

Terbutaline caused an increase in NK cell activity (Fig. 1), which started within 30 min and lasted for less than 2 h. This increase was statistically significant in all experiments except No. 3. PEF-rates were stable in all patients

Fig. 1. Individual diagrams showing the effect of 7 µg/kg terbutaline and placebo on NK cell activity. The *P*-value shows the statistical significance of the terbutaline-induced increase in NK activity compared with the control day values at the corresponding times. The referral number of experiment is shown in the upper left corner.

throughout the experiments, with the exception of 4 patients who showed a bronchodilation between 15 and 20 % compared with pretreatment values after the injection of terbutaline. The changes in NK activity did not show significant correlations with the changes in peak flow rates in the patients.

DISCUSSION

The results of this study show that a single dose of the beta-agonist terbutaline transiently stimulates peripheral blood NK activity *in vivo*.

Terbutaline has been shown to cause an increase in plasma cyclic AMP of more than 100 % within 30 min of subcutaneous injection and after inhalation, both in asthmatics (2) and in nonasthmatic volunteers (3). The similar kinetics of the rise in plasma cyclic AMP and the boost in natural cytotoxicity suggest that the immunological consequences of terbutaline injection detected in this work are due to the elevated levels of cyclic AMP. Our results therefore support the findings of Rola-Pleszczynski and co-workers, who have shown that a short exposure to theophylline and vasoactive intestinal peptide (VIP) stimulates NK cells (9, 10), whereas longer exposure times may be inhibitory to NK activity. In addition to the exposure time, the inhibition of cytotoxicity may also be dependent on the dosage of the modulating drugs. It has been shown, for example, that the NK-inhibitory concentrations of VIP are somewhat higher than the stimulatory ones (10).

We have previously reported that specific bronchial challenge with pollen antigens is associated with an augmentation of NK activity, which coincides with bronchoconstriction (15). Since the kinetics of the NK cell stimulation after the specific bronchial challenge and after terbutaline injection are similar, it is possible that the stimulation of NK activity during antigen exposure is at least partially due to endogenous adrenaline. However, other factors, particularly leukotriene B₄ may also be involved in the augmentation of cytotoxicity after specific challenge.

In our investigation, the spontaneous variation in the degree of bronchial obstruction was

negligible. Only 4 of our patients showed significant bronchodilation after the injection of terbutaline. These patients' NK activity did not differ significantly from that of the other patients.

In a previous study (13) we found increased NK activity in untreated asthmatics. Since results from different assays cannot be directly compared, and since reference samples from control donors were not included in the present study, the baseline NK activity of our patients cannot be classified as being increased or normal. Also our results do not answer the question whether patients with high basal NK activity react differently to terbutaline than those with low basal activity.

Beta-agonists are important drugs in the maintenance treatment of bronchial asthma. It has been shown that the basal cAMP concentration in lymphocytes from asthmatics and also the response to the cAMP-inducing action of isoproterenol are lower in asthmatics than in normals (4, 8). This alteration was most marked at the time of severe asthma and returned to normal during clinical remission (8). The response to beta-agonists also decreased with continuous medication (7). On the basis of the available *in vitro* data (5, 6, 9, 10, 14) it can be expected that the stimulatory effect of cAMP-inducing drugs on NK activity becomes less pronounced during regular daily treatment. Further studies need to be done to shed light on the clinical significance of the possible fluctuation of NK cell activity during long-term treatment of asthma.

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