

Effect of Betamethasone on the Dual Reaction to Anti-Human IgE in Man: Influence of Time Interval between Administration of Drug and Anti-IgE

R. GRÖNNEBERG and K. STRANDBERG

Dept. of Thoracic Medicine, Section of Allergology, Karolinska Hospital, Stockholm and
Section of Clinical Pharmacology, University Hospital, Uppsala, Sweden

The influence of the time interval between administration of a glucocorticoid, betamethasone, and anti-IgE on the effect of the drug on the early and late phase response (LPR) to anti-IgE was examined in 38 volunteers in a randomized controlled study. The immediate flare and wheal responses to intradermally (i.d.) injected anti-IgE were inhibited by approximately 20 and 30 % respectively by a 10 day i.d. pretreatment period with repeated 50 µg doses (total 300 µg) of betamethasone ($P < 0.01$), whereas responses to histamine were not influenced. A single i.d. injection of 50 µg betamethasone 24 h prior to anti-IgE challenge attenuated the wheal response ($P < 0.05$), whereas injection of the drug 2 h prior to and together with anti-IgE had no influence on the early response. Corresponding LPRs were reduced approximately 30 % throughout the observation period of 1-24 h ($P < 0.01$). Injecting the drug i.d. 30 min following anti-IgE challenge had no significant influence on the total observation period (1-24 h) of the LPR but antagonized the LPR by 35 % at 6-24 h ($P < 0.01$). It is concluded that glucocorticoids inhibit IgE-dependent LPRs. When extending the topical pretreatment period with the compounds also the early part of the allergic reaction is attenuated.

Key words: anti-IgE; dual reaction; early reaction; glucocorticoid; late phase reaction.

Accepted for publication 8 October 1984

CLINICAL ASPECTS

A late phase response (LPR) in airways following allergen challenge is a common clinical phenomenon. The reaction has certain counterparts in the LPR evoked by allergen and anti-IgE in skin. In volunteers the glucocorticoid betamethasone injected intradermally (i.d.) shortly before and together with anti-IgE antagonized the LPR with no consistent effect on the early reaction. A 10 day i.d. high-dose pretreatment period attenuated the early response as well. Notably, betamethasone antagonized the LPR also when administered shortly after anti-IgE challenge. This accords with the clinical observation of a beneficial action by glucocorticoids on late sustained inflammatory reactions when given after allergen challenge.

The pathogenesis of the early and late phase allergic response (LPR), the so-called dual reaction, is generally believed to involve an IgE-dependent release of mast cell-derived mediators with the subsequent activation of a biochemical cascade reaction leading to the expression of an LPR (10). When administered

together with or shortly prior to an allergen, glucocorticoids are potent inhibitors of the LPR without a clear-cut inhibitory effect on the early reaction (9, 14, 17, 19, 21, 25). In analogy we showed a marked anti-LPR effect but no influence on the early response by 50 µg betamethasone given intradermally together with

anti-human IgE in healthy subjects (14). The purpose of the present investigation was to further study the time dependency of the anti-allergic action of betamethasone by systematically varying the time interval of injection between the drug and anti-IgE.

MATERIAL AND METHODS

Antiserum and chemicals

Anti-human IgE was kindly supplied by Professor S. Gunnar O. Johansson, Department of Clinical Immunology, Karolinska Hospital, Stockholm, Sweden. The antiserum was raised in rabbits against Fc fragments from a human IgE myeloma protein. The serum was precipitated and washed with 18% Na₂SO₄, dissolved and dialysed against sodium chloride (0.15 mol/l) to 1/20 (v/v) containing 0.02 g/l specific antibody activity, passed through a millipore filter (0.45 µm) and stored at +4°C. The anti-IgE, histamine dihydrochloride, normal rabbit IgG diluted 1/10 (v/v) and betamethasone phosphate (Betapred®, Glaxo Laboratories Ltd., Greenford, Middlesex, England) were taken on the day of each experiment and further diluted with physiological saline containing 0.3% phenol.

Skin testing

Skin testing was performed in a total of 38 healthy volunteers (36 females, 2 males), 17–59 years of age (mean 31), and on no medication. Anti-IgE diluted 1/330 (v/v) and betamethasone, 50 µg (2.5 × 10⁻³ mol/l), were injected intradermally (i.d.) with a micrometer and a tuberculin syringe respectively, with various time intervals, in 7–10 subjects in each of three trials, i.e. the same test subjects did not take part on all occasions. Betamethasone or saline (control) in 30 µl was randomly injected in the volar aspect of the forearm 24 and 2 h prior to and together with anti-IgE. All injection sites were carefully outlined with a marking pen to enable later injection with anti-IgE. In another trial the pretreatment period was extended to 10 days. Betamethasone or saline in 50 µl were injected

repeatedly into two sites on six occasions with 24–72 h intervals between the injections. Challenge with anti-IgE or 5 µg histamine followed 24 h after the last injection of betamethasone or saline. Administration of the drug or saline in 100 µl 0.5 h subsequently to injection of anti-IgE was performed by i.d. injection into the anti-IgE injection site as well as by subcutaneous infiltration of the whealing in four directions. Normal rabbit IgG diluted 1/330 (v/v) was also administered to each test subject for specificity reasons.

Early flare and wheal reactions were outlined with a marking pen 15 min after injection of anti-IgE and traced on a transparent film for later planimetric measurement. Two perpendicular diameters (D × d) of the swelling reaction (LPR) to anti-IgE were measured at defined time-intervals up to 24 h by the test subjects themselves after thorough instructions. Measurements of early reactions in the 10-day pretreatment trial and LPRs at 6 h in all trials were made by independent observers. These observers and the test subjects were informed about the aim of the study but not of the order of injection of the different compounds.

The size of the early and late reactions to anti-IgE (mm² and mean arithmetic diameter) at drug-treated and control sites was compared in each individual, and differences for early reactions evaluated by paired Student's t-test, while corresponding data for LPRs were analysed by the aid of three-way analysis of variance and rank correlation. All subjects gave informed consent prior to participation in the study which was approved by the Karolinska Hospital Ethical Committee.

RESULTS

Early reactions. The influence on the early reaction to anti-IgE by betamethasone injected i.d. at various time-intervals in relation to anti-IgE is shown in Fig. 1. Betamethasone injected repeatedly i.d. during 10 days with a total dose of 300 µg had no influence on histamine-induced responses (not shown) but inhibited the flare and wheal responses to anti-IgE by 17 and 28% respectively ($P < 0.01$). Betametha-

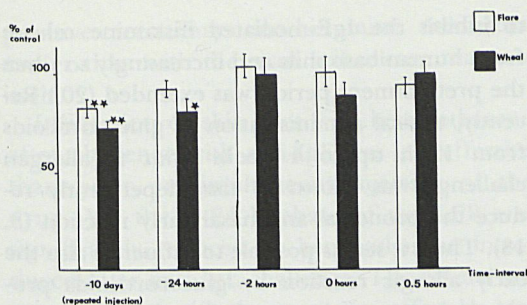
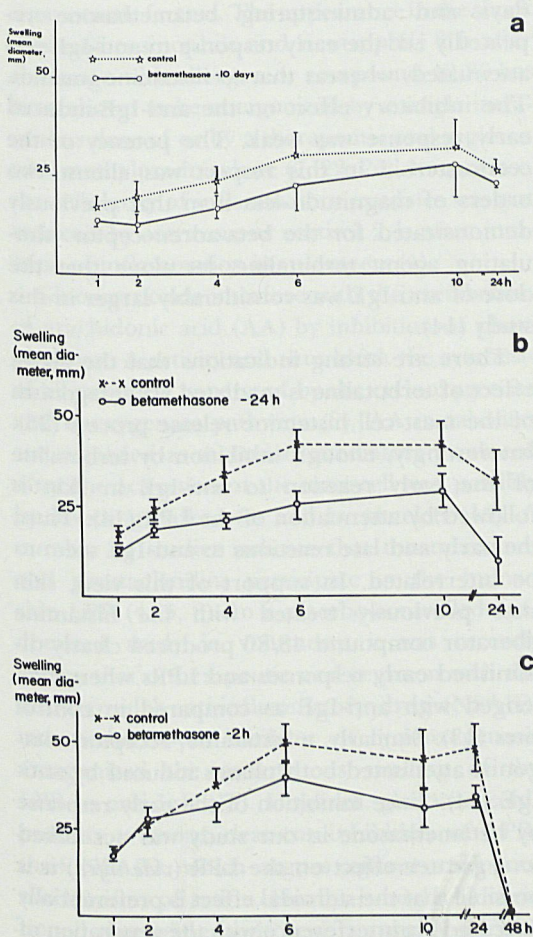


Fig. 1. Effect on flare and wheal responses to anti-IgE 1/330 (v/v), expressed as % of control, by i.d. betamethasone, 50 μ g, injected repeatedly (total dose 300 μ g) for 10 days prior to anti-IgE (-10 d) and as a single dose injected 24 (-24 h) and 2 h (-2 h) prior to, concomitantly with (0 h) and 0.5 h after (+0.5 h) anti-IgE challenge. Mean $\pm$ SEM, $n = 7$ in the -10 d trial and $n = 8$ in other trials. *, **Significant inhibition compared to control ($P < 0.05$ is denoted by * and $P < 0.01$ by ** respectively).



sone injected i.d. 24 h prior to anti-IgE attenuated the wheal response by 20% ($P < 0.05$) but had no significant effect on the flare response. Injecting the drug 2 h prior to or together with anti-IgE had no effect on the early reaction whatsoever.

Late phase reaction. Fig. 2 demonstrates the effect on the corresponding LPRs by i.d. betamethasone, 50 μ g. Administration of the drug 10 days, 24 h or 2 h prior to and together with anti-IgE produced an inhibition of 27 ± 9 , 33 ± 5 , 18 ± 5 and $35 \pm 8\%$ respectively (mean $\pm$ SEM) of the LPRs at 1-24 h as compared with control reactions. There was no significant time dependence of the anti-LPR-effect when comparing the data for the various treatment periods.

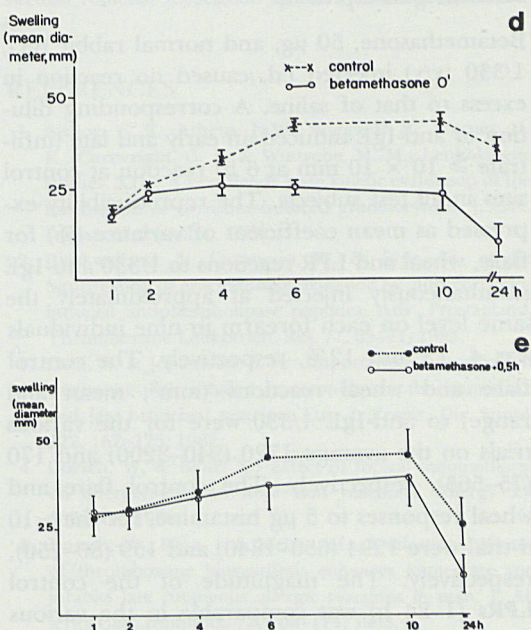


Fig. 2. Effect on LPR (arithmetic mean diameter, mm) to anti-IgE 1/330 (v/v) by betamethasone, 50 μ g, injected i.d. (mean $\pm$ SEM). a) 10 days prior to anti-IgE challenge ($n = 7$); b) 24 h prior to anti-IgE ($n = 10$); c) 2 h prior to anti-IgE ($n = 8$); d) together with anti-IgE ($n = 8$); e) 30 min after anti-IgE ($n = 8$).

Infiltration with betamethasone 0.5 h subsequently to anti-IgE had no influence on the 1–24 h observation period but antagonized the LPR by $33\% \pm 11$ ($P < 0.01$) at 6–24 h following anti-IgE injection. Previous and concomitant administration of drug and anti-IgE produced more marked anti-LPR-effects than subsequent (+0.5 h) injection of betamethasone ($P < 0.001$, $P < 0.05$ and $P < 0.001$ respectively) whereas 10 days pretreatment produced no additional antagonism. The anti-LPR-effect of 10 days pretreatment with betamethasone correlated with the degree of inhibitory influence of the drug on the wheal response ($P < 0.01$, rank correlation) but not with the corresponding effect on the flare response.

Methodological aspects

Betamethasone, 50 µg, and normal rabbit IgG, 1/330 (v/v) injected i.d. caused no reaction in excess to that of saline. A corresponding dilution of anti-IgE induced an early and late (infiltrate $\geq 10 \times 10$ mm at 6 h) reaction at control sites in all test subjects. The reproducibility expressed as mean coefficient of variance (%) for flare, wheal and LPR reactions to 1/330 anti-IgE simultaneously injected at approximately the same level on each forearm in nine individuals was 4, 12 and 12%, respectively. The control flare and wheal reactions (mm², mean and range) to anti-IgE 1/330 were for the various trials on the average 1720 (940–3200) and 170 (75–565), respectively. The control flare and wheal responses to 5 µg histamine, for the –10 d-trial were 1294 (650–1840) and 159 (80–250), respectively. The magnitude of the control LPRs (1–24 h) was comparable in the various trials except for the –10 d-trial where the LPR was 20–35% smaller than in the other ones ($P < 0.01$).

DISCUSSION

Glucocorticoids are potent inhibitors of the LPR in human skin and airways (10, 14, 17, 19, 25). *In vitro* glucocorticoids have been demonstrated

to inhibit the IgE-mediated histamine release from human basophils and increasingly so when the pretreatment period was extended (20). Recently, topical administration of glucocorticoids from 12 h up to 4 weeks prior to allergen challenge was shown to time-dependently reduce the bronchial and nasal early reaction (3, 18). Thus, it seems possible to influence also the early allergic reaction by glucocorticoids provided they are given well in advance of the allergen.

In harmony with previous *in vivo* data on allergen and anti-IgE induced reactions (9, 14, 17, 19, 21, 25) we found no clear attenuation of the early but significant inhibition of the late part of the reaction to anti-IgE administering betamethasone in a single dose 24 h or 2 h prior to or concomitantly with anti-IgE. However, when extending the pretreatment period to 10 days and administering betamethasone repeatedly i.d. the early response to anti-IgE was attenuated, whereas that to histamine was not. The inhibitory effect on the anti-IgE-induced early response was weak. The potency of the corticosteroid in this respect was almost two orders of magnitude less than that previously demonstrated for the beta-adrenoceptor stimulating agent, terbutaline, let alone that the dose of anti-IgE was considerably larger in this study (14).

There are strong indications that the above effect of terbutaline is mediated via antagonism of the mast cell histamine release process (23). Interestingly, enough inhibition by terbutaline of the early reaction to anti-IgE in skin is followed by attenuation of the LPR (14). Thus, the early and late reactions to anti-IgE seem to be interrelated. In support of this view, skin sites previously treated with the histamine liberator compound 48/80 produced clearly diminished early responses and LPRs when challenged with anti-IgE as compared to control sites (13). Similarly, a histamine₁-receptor antagonist attenuated both phases induced by anti-IgE (14). Since inhibition of the early response by betamethasone in our study was not linked to a greater effect on the LPR (*vide infra*) it is possible that the steroidal effect is preferentially executed via interference with the generation of

putative secondary non-histamine mediators rather than with the mast cell release reaction itself.

Infiltration of skin sites with betamethasone after challenge with anti-IgE was less effective than prior or simultaneous injection of the drug. Yet the 6–24 h phase of the LPR was significantly reduced by betamethasone introduced after anti-IgE challenge. The results are in accordance with the protective effect by oral prednisolone given shortly after allergen on the LPR to bronchial allergen inhalation (8). Our data are also in harmony with the clinical observation of therapeutic effects on various late sustained inflammatory phases by glucocorticoids administered after allergen challenge.

The anti-LPR action of glucocorticoids might be attributed to an effect on the recruitment of inflammatory cells as a result of the release of chemotactic factors. The appearance in the shock tissue of eosinophils and neutrophils 2 h after challenge with allergen or compound 48/80 has been shown to be antagonized by a single intravenous dose of or by a therapeutic oral regimen with methylprednisolone (7, 22, 26). In contrast, the cellularity of inflammatory exudates was not reduced by intravenous cortisol 2 h after initiation of inflammation in volunteers (1).

Glucocorticoids interfere with the metabolism of arachidonic acid (AA) by inhibition of phospholipase A activity and thus block the synthesis of all products generated by the cyclooxygenase and lipoxygenase pathways (2). AA metabolites are regarded as secondary mediators produced after the activation of the mast cell and possibly by invading leukocytes and by platelets (16). A number of studies indicate that thromboxanes and prostaglandins participate in the human skin LPR (4, 5, 6). In a placebo-controlled randomized study in volunteers it was recently shown that a therapeutic oral regimen with the non-steroidal anti-inflammatory drug (NSAID) indomethacin, an inhibitor of cyclooxygenase, antagonized the oedematous swelling of the LPR to anti-IgE (15). However, glucocorticoids produce a more clear-cut inhibition of LPRs than NSAID, which argues against analogous mechanisms of action. Finally, it might be mentioned that tranexamic acid, a compound with

antagonistic effect on the activation of the fibrinolytic and complement systems, was recently shown to attenuate the LPR to anti-IgE in healthy subjects, further underlining the complexity of the reaction (12). Glucocorticoids possess antagonistic effects on the secretion of plasminogen activators from macrophages and PMNs (11, 24).

It is concluded that the glucocorticoid betamethasone may inhibit the late phase response in human skin also when administered shortly after anti-IgE challenge. The early allergic reaction may be slightly attenuated by the drug when given topically well in advance of anti-IgE.

ACKNOWLEDGEMENTS

This investigation was kindly supported by grants from the Foundation of King Gustaf V:s 80th Anniversary and the Swedish National Association against Heart and Chest Diseases.

REFERENCES

1. Bishop, C. R., Athens, J. W., Boggs, D. R., Warner, H. R., Cartwright, G. E. & Wintrobe, M. M.: Leukokinetic studies. XIII. A non-steady state kinetic evaluation of the mechanism of cortisone-induced granulocytosis. *J. Clin. Invest.* 47, 249–260, 1968.
2. Blackwell, G. J., Carnuccio, R., Di Rosa, M., et al.: Suppression of arachidonate oxidation by glucocorticoid-induced antiphospholipase peptides. *Adv. Prostagland. Thromboxane Leukotrien. Res.* 11, 65–71, 1983.
3. Dahl, R. & Johansson, S.-Å.: Importance of duration of treatment with inhaled budesonide on the immediate and late bronchial reaction. *Eur. J. Respir. Dis. Suppl.* 122, 167–175, 1982.
4. Dorsch, W. & Baur, X.: Effect of topical indomethacin on allergen-induced dual skin reactions. *Allergy* 35, 503–508, 1980.
5. Dorsch, W., Ring, J. & Melzer, H.: A selective inhibitor of thromboxane biosynthesis enhances immediate and inhibits late cutaneous allergic reactions in man. *J. Allergy Clin. Immunol.* 72, 168–174, 1983.
6. Dorsch, W., Ring, J., Reimann, H. J. & Geiger, R.: Mediator studies in skin blister fluid from patients with dual skin reactions after intradermal allergen injection. *J. Allergy Clin. Immunol.* 70, 236–242, 1982.
7. Dunskey, E. H., Atkins, P. C. & Zweiman, B.: Histologic responses in human skin test reactions to ragweed. IV. Effects of a single i.v. injection of steroids. *J. Allergy Clin. Immunol.* 59, 142–146, 1977.
8. Fairfax, A. J.: Inhibition of the late asthmatic response to house dust mite by non-steroidal anti-inflammatory drugs. *Prostaglandins, Leukotriens. Med.* 8, 239–248, 1982.

9. Galant, S. P., Bulloch, J., Wong, D. & Maibach, H. I.: The inhibitory effect of anti-allergy drugs on allergen and histamine induced wheal and flare response. *J. Allergy Clin. Immunol.* 51, 11–21, 1973.
10. Gleich, G. J.: The late phase of the immunoglobulin E-mediated reactions: a link between anaphylaxis and common allergic disease? *J. Allergy Clin. Immunol.* 70, 160–169, 1982.
11. Granelli-Piperno, A., Vassalli, J.-D. & Reich, E.: Secretion of plasminogen activator by human polymorphonuclear leukocytes. Modulation by glucocorticoids and other effectors. *J. Exp. Med.* 146, 1693–1706, 1977.
12. Grönneberg, R.: Inhibition of the late phase reaction to anti-human IgE in man by oral tranexamic acid. *Allergy* 39, 115–118, 1984.
13. Grönneberg, R.: Inhibition of the late phase reaction to anti-IgE by previous mast cell activation with compound 48/80. *Allergy* 39, 119–123, 1984.
14. Grönneberg, R., Strandberg, K., Stålenheim, G. & Zetterström, O.: Effect in man of anti-allergic drugs on the immediate and late phase cutaneous allergic reactions induced by anti-IgE. *Allergy* 36, 201–208, 1981.
15. Grönneberg, R. & Zetterström, O.: Inhibition of the late phase response to anti-IgE in humans by indomethacin. *Allergy* 40, 36–41, 1985.
16. Moncada, S., Flower, R. J. & Vane, J. R.: Prostaglandins, prostacyclin and thromboxane A₂. In Goodman, L. & Gilman, A. (eds.): *The pharmacological basis of therapeutics*, 6th ed., pp. 668–681. MacMillan Publ. Co., New York, 1980.
17. Pepys, J., Turner-Warwick, M., Dawson, P. L. & Hinson, K. F. W.: Arthus (type III) skin test reactions in man. Clinical and immunological features. In Rose, B., Richter, M., Sebon, A. & Frankland, A. W. (eds.): *Allergology. Excerpta Medica International Congress Series No. 162*, pp. 221–235. Amsterdam, 1968.
18. Pipkorn, U.: The effect of budesonide on the immediate reaction to allergen challenge – a rhinomanometric study. *Eur. J. Respir. Dis. Suppl.* 122, 185–191, 1982.
19. Poothullil, J., Umemoto, L., Dolovich, J., Hargreave, F. E. & Day, R. P.: Inhibition by prednisone of late cutaneous allergic responses induced by antiserum to human IgE. *J. Allergy Clin. Immunol.* 57, 164–167, 1976.
20. Schleimer, R. P., Lichtenstein, L. M. & Gillespie, E.: Inhibition of basophil histamine release by anti-inflammatory steroids. *Nature* 292, 454–455, 1981.
21. Slott, R. I. & Zweiman, B.: A controlled study of the effect of corticosteroids on immediate skin test reactivity. *J. Allergy Clin. Immunol.* 54, 229–234, 1974.
22. Slott, R. I. & Zweiman, B.: Histological studies of human skin test responses to ragweed and compound 48/80. II. Effects of corticosteroid therapy. *J. Allergy Clin. Immunol.* 55, 232–240, 1975.
23. Ting, S., Zweiman, B. & Lavker, R.: Terbutaline modulation of human allergic skin reactions. *J. Allergy Clin. Immunol.* 71, 437–441, 1983.
24. Vassalli, J. D., Hamilton, J. & Reich, E.: Macrophage plasminogen activator: modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors and cyclic nucleotides. *Cell* 8, 271–281, 1976.
25. Zetterström, O.: Dual skin reactions and serum antibodies to subtilisin and *Aspergillus fumigatus* extracts. *Clin. Allergy* 8, 77–91, 1978.
26. Zweiman, B., Slott, R. I. & Atkins, P. C.: Histologic studies of human skin test responses to ragweed and compound 48/80 III. Effects of alternate day steroid therapy. *J. Allergy Clin. Immunol.* 58, 657–663, 1976.

Address:

R. Grönneberg, M.D.
Dept. of Thoracic Medicine
Section of Allergology
Karolinska Hospital
104 01 Stockholm
Sweden

This document is a scanned copy of a printed document. No warranty is given about the accuracy of the copy. Users should refer to the original published version of the material.