

An in vitro comparison of commonly used topical glucocorticoid preparations

Cristiana Stellato, MD, PhD,^a Jun Atsuta, MD, PhD,^b Carol A. Bickel, MS,^a and Robert P. Schleimer, PhD^a *Baltimore, Md, and Tsu Mie, Japan*

Background: Glucocorticoids (GC) are potent inhibitors of peripheral blood eosinophil, basophil, and airway epithelial cell function.

Objectives: We compared in vitro the inhibitory activity of synthetic GC used for topical treatment in asthma and rhinitis on basophil histamine release (HR), eosinophil viability, and expression of vascular cell adhesion molecule-1 (VCAM-1) in the human bronchial epithelial cell line BEAS-2B.

Methods: Cells were treated for 24 hours with increasing concentrations (range 10^{-13} to 10^{-6} mol/L) of fluticasone propionate (FP), mometasone furoate (MF), budesonide (BUD), beclomethasone dipropionate (BDP), triamcinolone acetonide (TAA), hydrocortisone (HC), or dimethyl sulfoxide diluent before challenge. HR was measured by a fluorometric assay, viability of purified eosinophils was assessed by erythrosin B dye exclusion, and expression of VCAM-1 was measured by flow cytometry.

Results: GC induced a concentration-dependent inhibition of anti-IgE-induced HR. Maximum inhibition ranged from 59.7% to 81%, with a rank order of GC potency of $FP > MF > BUD > BDP \equiv TAA \gg HC$. Three-day treatment of eosinophils with GC concentration-dependently inhibited IL-5-induced eosinophil viability, with a rank of potency almost identical to that observed with basophil HR. The rank order of potency of GC for inhibition of the expression of VCAM-1 in BEAS-2B cells was $MF \equiv FP \gg BUD > TAA > HC \equiv BDP$. Inhibitory concentration of 50% values revealed that epithelial cells were the most sensitive and eosinophils were the least sensitive.

Conclusions: These data, combined with information on pharmacodynamics of these drugs in vivo, may be useful in estimating GC local anti-inflammatory effects. (*J Allergy Clin Immunol* 1999;104:623-9.)

Key words: Glucocorticoids, basophil, eosinophil, epithelial cells, allergy

Glucocorticoids (GC) have been the most potent and effective drugs for the treatment of severe asthma since their discovery and synthesis in the late 1940s. Over the last 2 decades the use of potent inhaled GC preparations, which are metabolized rapidly once absorbed, has expanded widely. For many physicians treating asthma,

Abbreviations used

BDP:	Beclomethasone dipropionate
17-BMP:	Beclomethasone-17-monopropionate
BUD:	Budesonide
DMEM:	Dulbecco's modified Eagle's medium
DMSO:	Dimethyl sulfoxide
β -E:	β -Estradiol
FP:	Fluticasone propionate
GC:	Glucocorticoid
HC:	Hydrocortisone
HR:	Histamine release
IC ₅₀ :	Concentration at which each GC produced 50% inhibition
MF:	Mometasone furoate
PIPES:	Piperazine- N,N'-bis-(2-ethanesulfonic acid)
TAA:	Triamcinolone acetonide
VCAM-1:	Vascular cell adhesion molecule-1

inhaled GCs have now become a drug of first choice for even mild to moderate asthma because of the relative safety and clear efficacy of these compounds. Although a considerable amount is known about the mechanism of GC actions as anti-inflammatory drugs in general and for the treatment of asthma, relatively few studies have been performed to compare the available inhaled GC preparations that are being widely used.^{1,2} It is not a certainty that all GC will behave similarly, either in vivo or in different in vitro cellular systems. Variation in lipid solubility, receptor affinity, binding to lung tissue at sites other than the glucocorticoid receptor, and other factors can dramatically influence the potency and duration of action of GC in the lungs. We have recently shown that human lung tissue is active in metabolizing the endogenous GC hydrocortisone (HC) because of the presence of the enzyme 11 β -hydroxysteroid dehydrogenase in epithelial cells and perhaps other cells in the lung.^{3,4} Thus local metabolism may also influence the effectiveness of inhaled or endogenous GC in the airways. The purpose of this study was to compare commonly used inhaled GCs for efficacy in cellular assays with several important targets of GC action in the airway. The goal was to establish a rank order of potency of the following GCs in assays of steroid inhibition of basophil histamine release, eosinophil survival, and vascular cell adhesion molecule-1 (VCAM-1) expression in airway epithelial cells: mometasone furoate (MF), fluticasone propionate (FP), beclomethasone dipropionate (BDP), budesonide (BUD), triamcinolone acetonide (TAA), and HC. We set out to determine whether there are differences in their rank order of potency and concentration at which each

From the Johns Hopkins Asthma and Allergy Center, Baltimore, Md,^a and the Mie National Hospital, Tsu Mie, Japan.^b

Supported by grants from the National Institutes of Health and by gifts from Astra, Glaxo, and Schering-Plough.

Received for publication Dec 21, 1998; revised June 7, 1999; accepted for publication June 7, 1999.

Reprint requests: Cristiana Stellato, MD, PhD, Johns Hopkins Asthma and Allergy Center, 5501 Hopkins Bayview Circle, Baltimore, MD 21224-6801.

Copyright © 1999 by Mosby, Inc.

0091-6749/99 \$8.00 + 0 1/1/100691

GC produced 50% inhibition (IC_{50}) in these systems. We have observed that basophils are slightly more sensitive to GCs than eosinophils in the assays used and that airway epithelial cells are particularly sensitive to these inhaled GCs. The rank order of potency was relatively similar with all 3 assays.

MATERIAL AND METHODS

The following materials were purchased: piperazine- N,N'-bis-(2-ethanesulfonic acid) (PIPES), D-glucose, BSA, heparin, dimethyl sulfoxide (DMSO), erythrosin B (Sigma, St Louis, Mo), Percoll (Pharmacia, Uppsala, Sweden), RPMI containing 25 mmol/L HEPES, penicillin-streptomycin solution, FBS, L-glutamine (Gibco, Grand Island, NY), rhu-IL-5 (R&D Systems, Minneapolis, Minn), goat antihuman IgE (Kirkegaard and Perry Laboratories, Gaithersburg, Md), alcian blue dye (Fisher Scientific, Pittsburgh, Pa), Dynabeads (Dynal, Great Neck, NY), CD16 mAb (Immunotech, Westbrook, Me), Dulbecco's modified Eagle's medium (DMEM), HAM's F-12 medium, Ca^{++} - and Mg^{++} -free HBSS, Versene (Ca^{++} - and Mg^{++} -free HBSS with 0.02% EDTA), trace elements, phosphoethanolamine-ethanolamine, retinoic acid (Biofluids, Rockville, Md), insulin, HC, epidermal growth factor, and endothelial cell growth supplement (Collaborative Research, Bedford, Mass), cholera toxin, tri-iodothyronine (Sigma); heat-inactivated FCS, penicillin-streptomycin solution (Life Technologies, Gaithersburg, Md), recombinant human TNF- α (R&D Systems).

The following GCs were provided or purchased from the indicated sources: FP, BDP, and beclomethasone-17-monopropionate (17-BMP) (Glaxo); MF and TAA (Schering-Plough); BUD (Astra Draco); and HC and β -estradiol (β -E) (Sigma). Stock solutions of the GCs were prepared in DMSO at 0.1 mol/L concentration and stored at $-20^{\circ}C$. Buffers used for basophil histamine release were PAG (PIPES buffer [25 mmol/L] containing 110 mmol/L sodium chloride, 5 mmol/L potassium chloride, 0.003% BSA, 0.1% D-glucose), PAGCM (PAG containing calcium chloride 1 mmol/L and magnesium chloride 1 mmol/L).

Preparation, culture, and histamine release from basophil-enriched leukocyte suspensions

Venous blood was obtained from adult donors who had given informed consent and basophils were isolated with double-step Percoll gradients as previously described.⁵ Briefly, whole blood was anticoagulated with 0.1 mol/L EDTA and diluted with an equal volume of PIPES buffer, and the density was adjusted to 1.065 g/mL with 100% isotonic Percoll. The blood-Percoll mixture was layered over a cushion of Percoll of density 1.079 g/cm³ and then centrifuged at 400g for 15 minutes at $22^{\circ}C$. The cells were collected from the supernatant and from the plasma-Percoll interface, washed once in EDTA-sodium chloride solution to remove the Percoll and washed twice in PAG-EDTA, and the number and percentage of basophils in each fraction was determined. The mean percentage of basophils found in these fractions ranged from 5% to 12%, as determined by cell counts in Spiers-Levy chambers with use of Alcian blue.⁶ Cells were then washed and cultured in replicates of 1 mL in 24-well plates (Costar, Cambridge, Mass) at cell densities of 1 to 10×10^6 cells, depending on basophil purity, as described.⁷ Briefly, cells resuspended in RPMI 1640 media supplemented with 1% glutamine, 1% penicillin-streptomycin, and 10% FBS were cultured in various concentrations of GC or DMSO diluent for 24 hours at $37^{\circ}C$. Previous studies have shown that GCs have no effect on basophil viability during a 24-hour culture (not shown). Cells were then harvested, washed, and challenged with 0.1 μ g/mL of anti-IgE

(45 minutes, $37^{\circ}C$). At the end of the incubation, cell-free supernatants were assayed for histamine release with the automated fluorometric technique of Siraganian.⁸ Basophil histamine release was calculated as the percent of total cellular histamine after subtraction of spontaneous release (5%-8%).

Preparation, culture, and viability assessment of peripheral blood eosinophils

Human granulocytes were isolated from EDTA-anticoagulated venous blood of normal donors or patients with asymptomatic allergic rhinitis or asthma by Percoll (1.090 g/mL) gradient centrifugation at room temperature. After centrifugation, all procedures were carried out at $4^{\circ}C$ to minimize cell activation. Red blood cells were removed by hypotonic lysis followed by removal of CD16-positive cells (neutrophils) with an immunomagnetic bead technique.⁹ Eosinophil purity (on the basis of examination of Diff-Quik-stained cytocentrifugation preparations) was $99\% \pm 1\%$ and viability (on the basis of erythrosin B dye exclusion) was $99\% \pm 1\%$. Cells were resuspended in RPMI 1640 medium supplemented with 1% glutamine, 1% penicillin-streptomycin, 10% FBS, and IL-5 (0.01 ng/mL) and placed (in replicates of 100 μ L) in 96-well flat-bottom plates (Costar) at a cell density of 2.5×10^5 eosinophils per milliliter in the presence of various concentrations of GC, DMSO diluent, or medium alone for 3 days at $37^{\circ}C$. After the incubation period 70 μ L of supernatant was carefully removed, without disturbing the eosinophils settled at the bottom of the wells. Erythrosin B (2-3 μ L) was added to wells with gentle pipette mixing, and staining was allowed for precisely 5 minutes. A cell sample was then counted at $\times 40$ magnification. Viability of cells was expressed as percent of total cells counted.

Culture and challenge of BEAS-2B epithelial cells

BEAS-2B cells were a generous gift from Dr Curtis Harris (National Cancer Institute, Bethesda, Md). This cell line was derived from human bronchial epithelium transformed by an adenovirus 12-SV40 hybrid virus.¹⁰ These cells retain electron microscopic features of epithelial cells and show positive staining with antibodies to cytokeratin but do not form tight junctions¹¹ (data not shown). BEAS-2B cells were cultured in 25-cm² tissue culture flasks and maintained in F12/10 \times medium consisting of Ham's F12 nutrient medium containing penicillin (100 U/mL) and streptomycin (100 U/mL) and supplemented with insulin (5 μ g/mL), HC (10^{-7} mol/L), tri-iodothyronine (3.1×10^{-9} mol/L), cholera toxin (10 ng/mL), endothelial cell growth supplement (3.75 μ g/mL), epidermal growth factor (12.5 ng/mL), phosphoethanolamine-ethanolamine (5×10^{-6} mol/L), trace elements (1 \times), and retinoic acid (0.1 μ g/mL). Cells were used between passages 35 and 47 and were plated on 6-well cluster plates (Costar) and cultured for at least 48 hours in F12/DMEM medium (which lacks added HC) containing 5% heat-inactivated FCS, penicillin (100 U/mL), and streptomycin (100 U/mL) before harvesting.

Flow cytometric analysis

In the experiments assessing the inhibitory effects of glucocorticoids on VCAM-1 expression on BEAS-2B cells, cells were preincubated with increasing concentrations of GCs or an equivalent concentration of DMSO diluent for 24 hours and then incubated with TNF- α (10 ng/mL) for 24 hours. These methods are described in detail elsewhere.^{12,13} Cells were then washed 3 times with Ca^{++} - and Mg^{++} -free HBSS and treated for 10 minutes with Versene (Ca^{++} - and Mg^{++} -free HBSS containing 0.02% EDTA) without trypsin and then removed from plates by repeated pipetting. For each analysis 1×10^6 cells were incubated in 30 μ L of PBS/0.2% BSA containing

TABLE I. Concentration of glucocorticoids required for inhibition of basophil HR, eosinophil survival, and epithelial VCAM-1 expression

GC	Basophil HR (mol/L)	Eosinophil survival (mol/L)	BEAS-2B VCAM-1 expression (mol/L)
FP	7×10^{-11} *	2.5×10^{-10} †	4.6×10^{-12} ‡
MF	2×10^{-10}	7×10^{-10}	1.8×10^{-12}
BUD	5.9×10^{-10}	5.9×10^{-9}	3×10^{-10}
BDP	1×10^{-9}	3×10^{-8}	3×10^{-8}
TAA	3×10^{-9}	1×10^{-8}	6.3×10^{-10}
HC	1.5×10^{-7}	2.5×10^{-6}	1.1×10^{-8}

*IC₅₀ of HR.

†Concentration for half maximal reduction in eosinophil survival.

‡IC₅₀ of TNF- α -induced VCAM-1 expression.

saturation concentrations of each mAb and 4 mg/mL of human IgG (to reduce nonspecific binding) on ice for 30 minutes, as previously described.^{14,15} mAb for detection of intercellular adhesion molecule-1 was RR1 (AMAC, Westbrook, Me) and for VCAM-1 was BBIG-V1 (R&D Systems). The cells were washed, resuspended in saturating amounts of fluorescein-conjugated goat F(ab')₂ anti-mouse IgG antibody (Bio Source, Camarillo, Calif) for another 30 minutes, and then washed again. Negative staining with propidium iodide (2 μ g/mL) and a combination of scatter characteristics were used to identify a uniform population of viable cells. Fluorescence was measured with an EPICS Profile II flow cytometer (Coulter Electronics, Hialeah, Fla) and was expressed as percent of control IgG mean fluorescence intensity by comparison to control staining with an irrelevant isotype-matched mouse mAb. For each sample, at least 5000 events were collected.

Statistical analysis

Analysis of data was performed with use of Statview II software (Abacus Concepts, Berkeley, Calif) on a Macintosh IIsi computer. Data are expressed as mean \pm SEM. Statistical analysis between groups was performed with the ANOVA test with a post-hoc analysis (Fisher PLSD test). A *P* value <.05 was considered significant.

RESULTS

Glucocorticoids are first-line drugs in the therapy of airway allergic diseases such as asthma and rhinitis.² The inflammatory cells infiltrating the site of allergic reactions from peripheral blood, such as eosinophils and basophils, are all targets of the anti-inflammatory activity of GCs.¹⁶ These compounds are effective inhibitors of IgE-mediated mediator release by basophils.^{7,16} It has been shown that several GCs used by oral administration exert their inhibitory effect on basophils with a rank of potency that parallels that found in vivo.¹⁴ GCs also exert profound inhibitory effects on the recruitment, activation, and survival of eosinophils.¹⁷ In the current in vitro study we compared the effect of several synthetic GCs currently used for topical treatment in asthma and rhinitis on basophil mediator release, eosinophil viability, and epithelial cell activation. Among the resident cells in the airways, epithelial cells are a particularly important target of GC action.¹⁸

Fig 1 shows the results of a series of experiments (*n* = 2-6) in which basophil-enriched cell suspensions were

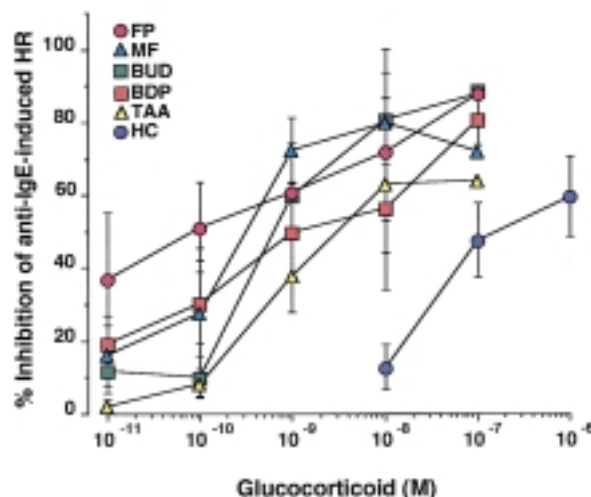


FIG 1. Inhibition of basophil histamine release with GC. Basophils were cultured for 24 hours in presence of indicated GC or DMSO diluent and subsequently challenged with 0.1 μ g/mL anti-IgE. Data shown are mean \pm SEM inhibition of histamine release (HR) from 2 to 6 experiments; percent HR induced by anti-IgE in DMSO-treated cells was 36.1% \pm 12.2%.

incubated for 24 hours in the presence of increasing concentrations (10^{-11} to 10^{-6} mol/L) of the indicated GC or DMSO diluent before challenge with 0.1 μ g/mL anti-IgE. Treatment with GC induced a concentration-dependent inhibition of anti-IgE-induced histamine release, with maximum inhibition ranging from 59.7%, achieved by HC at 10^{-6} mol/L, to 81%, induced by BDP at 10^{-7} mol/L. Because a maximum effect was not reached in every experiment, for each GC we arbitrarily chose a defined net effect—50% inhibition of histamine release—calculated on the mean \pm SEM of all experiments to express the concentration at which each GC produced IC₅₀. These values are indicated in Table I, showing a rank order of potency of FP > MF > BUD > BDP \approx TAA >> HC.

We next compared the ability of the various GCs to affect IL-5-sustained eosinophil survival by assessing eosinophil viability by erythrosin B dye exclusion after culture in the presence of GC. In a series of preliminary experiments, to identify a concentration of IL-5 able to prolong eosinophil survival without overriding the inhibitory activity of GC, we cultured eosinophils for 3 days with a broad spectrum of IL-5 concentrations (0.002-5 ng/mL) in medium only, DMSO diluent, or 10^{-7} mol/L BUD. A suboptimal concentration of IL-5 (0.01 ng/mL) was chosen, at which both eosinophil viability and the inhibitory effect of the steroid were optimal (Fig 2). The presence of DMSO did not affect eosinophil viability (Fig 2). Subsequently, we performed a series of experiments assessing the effect of 3 days of treatment with increasing concentrations (10^{-13} to 10^{-6} mol/L) of the different GC on eosinophil viability (*n* = 6). As was the case for basophil HR, a concentration-dependent inhibition of eosinophil survival was observed (Fig 3). Because of the relationship between IL-5 and steroid, the drugs did not reduce viability by 100%. Because the

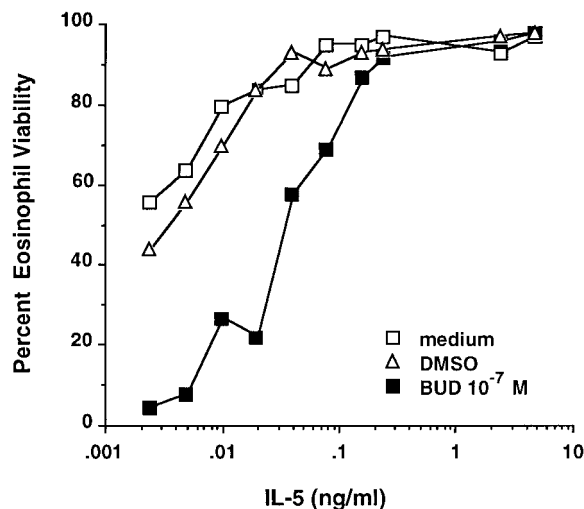


FIG 2. Titration of IL-5 concentration used to prolong eosinophil survival (representative of $n = 7$ for IL-5 titration). Eosinophils were incubated with the indicated concentrations of IL-5 for 3 days in the presence of medium alone, DMSO diluent, or BUD (10^{-7} mol/L). Eosinophil viability was assessed by erythrosin B dye exclusion. Viability of cells is expressed as percent of total cells counted.

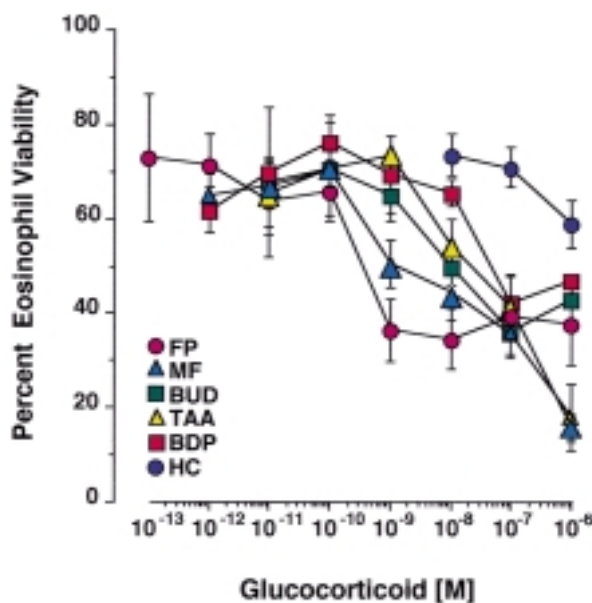


FIG 3. Inhibition of eosinophil survival by GC. Eosinophils were cultured for 3 days in medium containing 0.01 ng/mL rhu-IL-5 in presence of indicated concentrations of GC, DMSO diluent, or medium alone. Viability in DMSO-treated cells was $62\% \pm 10\%$. Results shown are mean \pm SEM of $n = 6$.

steroids had a maximal effect of approximately 50% reduction of viability, a group maximum inhibition was determined and the concentration of each steroid that inhibited survival by half of this value was interpolated. The rank order of potency among the GCs in this assay differed slightly from the basophil assay, notably for triamcinolone and beclomethasone. The rank order of potency observed was $FP > MF > BUD > TAA \approx BDP > HC$, as shown by the IC_{50} values displayed in Table I.

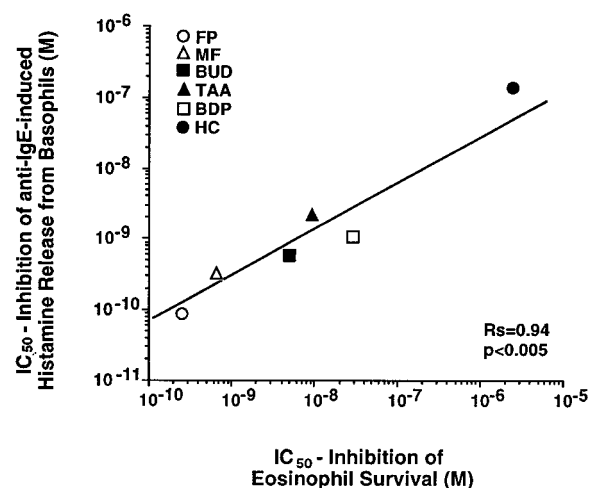


FIG 4. Correlation among potencies of GCs in inhibiting basophil HR and eosinophil viability.

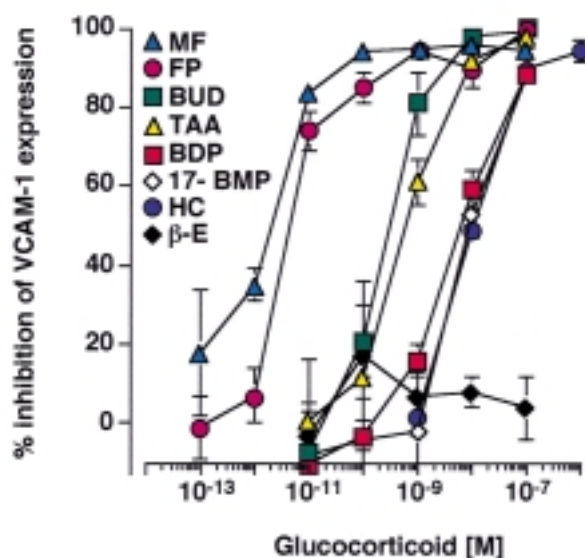


FIG 5. Inhibition of VCAM-1 expression on BEAS-2B epithelial cells. BEAS-2B cells were preincubated with various concentrations of GC for 24 hours before stimulation with 10 ng/mL of TNF- α for another 24 hours. Expression of VCAM-1 was measured by flow cytometry. Results shown are means \pm SEM of $n = 3$. (Reprinted from Atsuta J, Plitt J, Bochner BS, Schleimer RP. Inhibition of VCAM-1 expression in human bronchial epithelial cells by glucocorticoids. *Am J Respir Cell Mol Biol* 1999;20:643-50. Used with permission.)

Nevertheless, for the series of drugs, a positive correlation existed ($r_s = 0.94$, $P < .005$) between the potency observed in inhibiting basophil histamine release and eosinophil viability (Fig 4). In spite of the preservation of the relative rank order of potency, the basophil HR assay was more sensitive to all GCs than the eosinophil viability assay was, with a shift of sensitivity of 2- to 5-fold (Table I). Data in Fig 5 show previously published results from an assessment of the potency of the GC preparations as inhibitors of VCAM-1 expression induced by TNF- α in cultured BEAS-2B airway epithelial cells.¹³

Previous studies have shown that stimulation of BEAS-2B cells with TNF- α and other cytokines induces expression of the adhesion molecule VCAM-1.¹³ BEAS-2B cells were preincubated with various concentrations of GCs for 24 hours before stimulation with 10 ng/mL of TNF- α for another 24 hours. VCAM-1 expression was measured by flow cytometry. Data in Fig 5 show that all GCs tested inhibited VCAM-1 expression induced with 10 ng/mL TNF- α in a concentration-dependent manner. β -E, used as a control steroid, did not change TNF- α -induced VCAM-1 expression. The concentrations at which each GC produced IC₅₀ are listed in Table I, showing a rank order of potency MF \approx FP \gg BUD \approx TAA $>$ HC \approx BDP. The most potent of the GCs tested were MF and FP, both of which had IC₅₀s less than 10⁻¹¹ mol/L. BUD and TAA had intermediate potency, and HC, BDP, and the BDP metabolite 17-BMP (IC₅₀ = 9.2 \times 10⁻⁹ mol/L) were the least potent of the steroids tested. GC treatment had no effect on expression of intercellular adhesion molecule-1 (data not shown). Comparison of the potencies in the 3 assays revealed that BEAS-2B cells were the most sensitive, eosinophils were the least sensitive, and basophils had intermediate sensitivity to inhibitory effects for the GC (Fig 6). A correlation was found between the BEAS-2B assay and the basophil assay, but it did not reach statistical significance (r_s = 0.77, P = .072). A correlation was found between the potencies of the steroids in the BEAS-2B assay and the eosinophil assay (r_s = 0.88, P < .05).

DISCUSSION

The purpose of the current study was to further characterize commonly used inhaled GCs for their relative potency in several in vitro assays of anti-inflammatory action. It is now well established that GCs achieve their potent anti-inflammatory effect by modulating the function of multiple cell targets relevant in the establishment of allergic inflammation. Peripheral blood eosinophils and basophils are the effector cells selectively recruited within the tissue site of a chronic allergic reaction and during the late-phase response in experimental challenge models.¹⁹ Release of cytotoxic and proinflammatory mediators from these cells is believed to contribute to the structural damage of the airway mucosa seen in patients with respiratory allergies. Structural cells, such as airway epithelial cells, are also important sources of proinflammatory mediators, cytokines, and eosinophil-specific chemoattractants,²⁰ and they interact with inflammatory cells through several adhesion pathways.¹² Epithelial cells, as well as eosinophils and basophils, are GC sensitive.²¹ Although one important effect of GC is the inhibition of cytokine and chemokine production,¹¹ GCs also have direct effects on inflammatory cells, including inhibition of basophil histamine release, decrease in eosinophil survival, and suppression of epithelial cell activation, independent of their ability to suppress cytokine synthesis. We have compared inhaled steroids commonly used for the treatment of asthma for their abil-

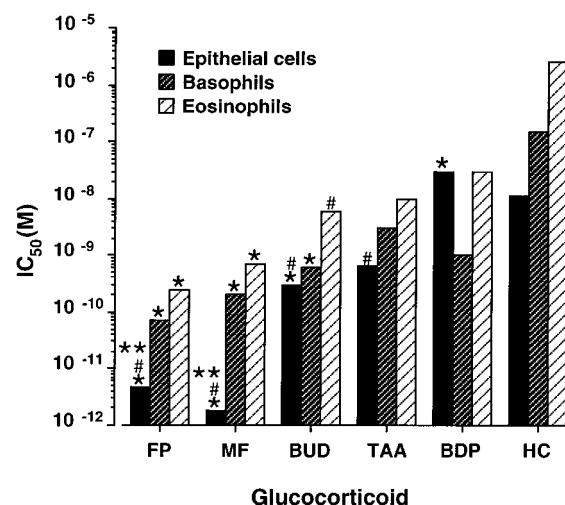


FIG 6. Comparison of IC₅₀ values obtained for various GCs for inhibition of basophil histamine release, eosinophil survival, and expression of VCAM-1 on bronchial epithelial cell line BEAS-2B. Asterisk, P < .05 compared with HC; pound sign, P < .05 compared with BDP; two asterisks, P < .05 compared with TAA.

ity to inhibit basophil histamine release, eosinophil survival, and epithelial activation for VCAM-1 expression. In a recent study, Spahn et al¹ determined the potencies of several topical GCs in inhibiting phytohemagglutinin-induced proliferation of PBMCs from asthmatic patients. The rank order of potency found by these investigators in the inhibition of lymphocyte activation (BUD $>$ TAA $>$ BDP $>$ HC) is almost identical to that found in this study in all 3 assays. Similarly, Umland et al²² report a very similar rank order of potency among topical GCs in their ability to suppress IL-4 and IL-5 production from peripheral blood lymphocytes (MF = FP $>$ BUD $>$ BDP \approx TAA). The use of the standard test for determining relative topical anti-inflammatory potency, the MacKenzie topical skin blanching test, as reported in the Second Expert Panel Report of the National Asthma Education and Prevention Program from the National Institutes of Health, established a rank order of potency (FP $>$ BUD $>$ BDP $>$ TAA)²³ very similar to that reported in our study.

The potency of several of the tested GCs, which included MF, FP, BDP, BUD, TAA, and HC, to inhibit basophil histamine release was severalfold greater than their potency in inhibiting eosinophil survival but lower than their actions on epithelial cells. The molecular explanation for this clear difference in potency in the 3 assays is not obvious from the presented data. One possible explanation for the relative resistance of the eosinophil assay is the presence of added cytokine, namely IL-5. Cytokines have been known for some time to be capable of antagonizing the action of GC in vitro. One of the first observations of this effect was the finding that the addition of IL-2 to lymphocyte proliferation assays could reverse the inhibitory effects of GCs on mitogenesis.^{15,24} In our early studies with human basophils we found that supernatants from activated T cells contained a factor that could antagonize the action

of GCs. We later found that this factor was the cytokine IL-3, which is a potent potentiator of basophil release of histamine and LT mediators.²⁵ In the presence of high concentrations of IL-3, the GC effect was lost entirely. Similarly, in eosinophil assays we found that high concentrations of GM-CSF could completely abrogate the inhibitory effects of GCs on eosinophil survival.^{26,27} This was confirmed by other laboratories studying eosinophil survival induced by IL-3 and IL-5 but not by IFN- γ .^{28,29} The possible molecular mechanism that underlies this may be extrapolated from studies on the molecular action of GC on transcription factor systems. These studies have shown that the GC receptor complex can physically interact with several transcription factors, including activator protein (AP)-1, and thereby block the activation of specific response elements for the AP-1 transcription factor (the so-called TPA-response element).³⁰ Conversely, however, the AP-1 transcription factor can, by virtue of the same physical interaction, prevent the GC receptor complex from interacting with its own receptor sites, including the glucocorticoid response element as well as a variety of transcription factors. Thus a strong elevation of transcription factors such as AP-1 could theoretically neutralize the GC receptor complexes in a given cell, rendering them incapable of suppressing the function of the cell. This type of acquired GC resistance at a cellular level has been described by Sher et al³¹ in lymphocyte systems where the combination of IL-2 and IL-4 can produce a cellular phenotype reminiscent of that observed in GC-resistant patients. The 3- to 5-fold difference in potency of GCs in inhibiting eosinophil survival compared with basophil histamine release may be related to such a mechanism.

The GCs studied were more potent at inhibiting epithelial cell activation and basophil histamine release than eosinophil survival. Nonetheless, the steroids with the highest binding affinity for the GC receptor³² were the most potent in inhibiting epithelial cell activation, basophil histamine release, and eosinophil survival. FP, MF, and BUD were all found to have particularly high potency, with IC₅₀s below the nanomolar range in the basophil assays. In the lung BDP is hydrolyzed to 17-BMP, which displays a GC receptor affinity 25 times higher than that of BDP.³³ The weak inhibition of VCAM expression obtained in our study with 17-BMP was therefore surprising. We are currently evaluating whether this was the result of further degradation of 17-BMP into inactive metabolites. The large differences in potency among inhaled glucocorticoids in vitro in suppressing VCAM-1 expression, eosinophil survival, or basophil histamine release is not reflected in vivo when the potency of these drugs in reversing symptomatic endpoints (eg, FEV₁, eosinophil number, or bronchial reactivity) is compared. It can be hypothesized that inhalation of any of these drugs will lead to transient saturation or near saturation of glucocorticoid receptors in epithelial cells and other airway cells. In such a case, other parameters, such as the residence time of the drug in the airways and the dissociation rate of the steroid from receptors, may determine clinical potency.

We thank Dr Donald MacGlashan, Jr, and Linda Friedhoff for helpful assistance in statistical analysis. We thank Dr Gunther Hochhaus for helpful discussion on data and Ms Bonnie Hebden for excellent secretarial assistance.

REFERENCES

- Spahn JD, Landwehr LP, Nimmagadda S, Surs W, Leung DYM, Szefer SJ. Effects of glucocorticoids on lymphocyte activation in patients with steroid-sensitive and steroid-resistant asthma. *J Allergy Clin Immunol* 1996;98:1073-9.
- Schleimer RP. Glucocorticosteroids: their mechanisms of action and use in allergic diseases. In: Middleton E, Reed CE, Ellis EF, Adkinson NF Jr, Yunginger JW, Busse W, eds. *Allergy: principles and Practice*. St Louis: Mosby; 1998. p 638-60.
- Schleimer RP. Potential regulation of inflammation in the lung by local metabolism of hydrocortisone. *Am J Respir Cell Mol Biol* 1991;4:166-73.
- Schleimer R, Kato M. Regulation of lung inflammation by local glucocorticoid metabolism—an hypothesis. *J Asthma* 1992;29:303-17.
- Warner JA, Reshef A, MacGlashan DW Jr. A rapid Percoll technique for the purification of basophils. *J Immunol Methods* 1987;105:107-10.
- Gilbert HS, Ornstein L. Basophil counting with a new staining method using alcian blue. *Blood* 1975;46:279-86.
- Schleimer RP, Lichtenstein LM, Gillespie E. Inhibition of basophil histamine release by anti-inflammatory steroids. *Nature* 1981;292:454-5.
- Siraganian RP. An automated continuous-flow system for the extraction and fluorometric analysis of histamine. *Anal Biochem* 1974;57:383-91.
- Hansel TT, DeVries JM, Iff T, Rihs S, Wandzilak M, Betz S, et al. An improved immunomagnetic procedure for the isolation of highly purified human blood eosinophils. *J Immunol Methods* 1991;145:105-10.
- Reddel RR, Ke Y, Gerwin BI, McMenamin MG, Lechner JF, Su RT, et al. Transformation of human bronchial epithelial cells by infection with SV40 or adenovirus-12 SV40-hybrid virus, or transfection via strontium phosphate coprecipitation with a plasmid containing SV40 early region genes. *Cancer Res* 1988;48:1904-9.
- Schleimer RP, Beck L, Schwiebert L, Stellato C, Bochner BS. Inhibition of inflammatory cell recruitment by glucocorticoids: cytokines as primary targets. In: Schleimer RP, Busse WW, O'Byrne P, editors. *Topical glucocorticoids in asthma: mechanisms and clinical actions*. New York: Marcel Dekker; 1997. p 203-38.
- Atsuta J, Sterbinsky SA, Schwiebert LM, Bochner BS, Schleimer RP. Phenotyping and cytokine regulation of the BEAS-2B human bronchial epithelial cell: demonstration of inducible expression of the adhesion molecule VCAM-1 and ICAM-1. *Am J Respir Cell Mol Biol* 1997;17:571-82.
- Atsuta J, Plitt J, Bochner BS, Schleimer RP. Inhibition of VCAM-1 expression in human bronchial epithelial cells by glucocorticoids. *Am J Respir Cell Mol Biol* 1999;20:643-50.
- Schleimer RP, MacGlashan DW Jr, Gillespie E, Lichtenstein LM. Inhibition of basophil histamine release by anti-inflammatory steroids, II: studies on the mechanism of action. *J Immunol* 1982;129:1632-6.
- Gillis S, Crabtree GR, Smith KA. Glucocorticoid-induced inhibition of T-cell growth factor production, II: the effect on the in vitro generation of cytolytic T-cells. *J Immunol* 1979;123:1632-8.
- Schleimer RP. Effects of glucocorticoids on inflammatory cells relevant to their therapeutic applications in asthma. *Am Rev Respir Dis* 1990;141:S59-69.
- Schleimer RP, Bochner BS. The effects of glucocorticoids on human eosinophils. *J Allergy Clin Immunol* 1994;94:1202-13.
- Schwiebert LM, Stellato C, Schleimer RP. The epithelium as a target of glucocorticoid action in the treatment of asthma. *Am J Respir Crit Care Med* 1996;154:S16-20.
- Bochner BS, Schleimer RP. Mechanisms responsible for preferential recruitment of human eosinophils and basophils during allergic inflammation. In: Kaplan AP, editors. *Immunomodulation of allergic diseases*. Pharmacia Allergy Research Foundation. Award Book. 1996. p 12-23.
- Proud D. The epithelial cell as a target and effector cell in airway inflammation. In: Holgate ST, Austen KF, Lichtenstein LM, Kay AB, editors. *Asthma: physiology, immunopharmacology, and treatment*. London: Academic Press; 1993. p 199-209.
- Stellato C, Schwiebert LM, Schleimer RP. Mechanisms of glucocorticoids

- teroid action. In: Barnes PJ, Grunstein MM, Leff A, Woolcock AJ, editors. *Asthma*. Philadelphia: Lippincott-Raven; 1997. p 1569-96.
22. Umland SP, Nahrebne DK, Razac S, Beavis A, Pennline KJ, Egan RW, et al. The inhibitory effects of topically active glucocorticoids on IL-4, IL-5, and interferon-gamma production by cultured primary CD4⁺ T cells. *J Allergy Clin Immunol* 1997;100:511-9.
23. National Asthma Education Program. Expert Panel Report 2: guidelines for the diagnosis and the management of asthma. Bethesda (MD): National Institutes of Health; 1997. Publication No.: 97-4051.
24. Lotta-Larsson E. Cyclosporin A and dexamethasone suppress T-cell responses by selectively acting at distinct sites of the triggering process. *J Immunol* 1980;124:2828-33.
25. Schleimer RP, Derse CP, Friedman BF, Gillis S, Plaut M, Lichtenstein LM, et al. Regulation of human basophil mediator release by cytokines, I: interaction with antiinflammatory steroids. *J Immunol* 1989;143:1310-7.
26. Lamas AM, Marcotte GV, Schleimer RP. Human endothelial cells prolong eosinophil survival: regulation by cytokines and glucocorticoids. *J Immunol* 1989;142:3978-84.
27. Lamas AM, Leon OG, Schleimer RP. Glucocorticoids inhibit eosinophil responses to granulocyte-macrophage colony-stimulating factor. *J Immunol* 1991;147:254-9.
28. Wallen N, Kita H, Weiler D, Gleich GJ. Glucocorticoids inhibit cytokine-mediated eosinophil survival. *J Immunol* 1991;147:3490-5.
29. Cox G, Ohtoshi T, Vanceri C, Denburg JA, Dolovich J, Gaudie J, et al. Promotion of eosinophil survival by human bronchial epithelial cells and its modulation by steroids. *Am J Respir Cell Mol Biol* 1991;4:525-31.
30. Karin M, Saatcioglu F. Negative transcriptional regulation by the glucocorticoid receptor is responsible for the antiinflammatory activity of glucocorticoids. In: Schleimer RP, Busse WW, O'Byrne PM, editors. *Inhaled glucocorticoids in asthma: mechanisms and clinical actions*. New York: Marcel Dekker; 1997. p 29-52.
31. Sher ER, Leung DYM, Surs W, Kam JC, Zieg G, Kamada AK, et al. Steroid-resistant asthma: cellular mechanisms contributing to inadequate response to glucocorticoid therapy. *J Clin Invest* 1994;93:33-9.
32. Edsbäcker S, Szeffler SJ. Glucocorticoid pharmacokinetics: principles and clinical applications. In: Schleimer RP, Busse WW, O'Byrne P, editors. *Inhaled glucocorticoids in asthma: mechanisms and clinical actions*. New York: Marcel Dekker; 1997. p 381-446.
33. Wurthwein G, Rohdewald P. Activation of beclomethasone dipropionate by hydrolysis to beclomethasone-17-monopropionate. *Biopharm Drug Dispos* 1990;11:381-94.