Development of fluticasone propionate and comparison with other inhaled corticosteroids

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Fluticasone propionate (FP) is a trifluorinated glucocorticoid based on the androstane nucleus. It was selected for development from structure-activity relationships (topical anti-inflammatory, cutaneous vasoconstriction, and hypothalamic-pituitary-adrenal axis suppression) of a series of 17β-carbothioates. FP is 3-, 300-, and 1000-fold more lipophilic than beclomethasone dipropionate, budesonide, and triamcinolone acetonide, respectively. FP has an absolute affinity (K_D) for the glucocorticoid receptor of 0.5 nmol/L and a relative receptor affinity 1.5-fold higher than beclomethasone-17-monopropionate (17-BMP) and mometasone furoate, 3-fold higher than budesonide, and 20-fold higher than flunisolide and triamcinolone acetonide. The rate of association of FP with the receptor is faster and the rate of dissociation slower than other corticosteroids. The resulting half-life of the FP active steroid-receptor complex is >10 hours, compared with approximately 5, 7.5, and 4 hours for budesonide, 17-BMP, and triamcinolone acetonide, respectively. FP has high selectivity for the glucocorticoid receptor, with little or no activity at other steroid receptors. FP is more potent than beclomethasone dipropionate, budesonide, triamcinolone acetonide, and mometasone furoate in inhibiting human T-cell migration and proliferation, inhibiting CD4+ T-cell cytokine and basophil histamine release, attenuating adhesion molecule expression, stimulating inflammatory cell apoptosis, and inducing cellular antiprotease release. In asthma patients, FP decreases the number of CD3+, CD4+, CD8+, and CD25+ T cells, mast cells, and eosinophils in bronchial biopsies, in addition to suppressing CD1a-dendritic and IgE+ cells and HLA-DR. FP, therefore, has a good pharmacologic profile for a topical steroid with increased intrinsic glucocorticoid potency and potent anti-inflammatory activity.
(J Allergy Clin Immunol 1998;101:S434-9.)

Key words: *Fluticasone propionate, inhaled corticosteroids, structure-activity relationships, asthma*

To exert anti-inflammatory activity, a corticosteroid molecule must penetrate the cellular membrane and demonstrate affinity for the steroid binding site on the glucocorticoid receptor (GR), leading to activation of the receptor.[1] Dimerization of the active steroid-receptor complex occurs, and this can then enter the nucleus, bind to glucocorticoid-responsive elements on a target gene, influence gene transcription, and either inhibit proinflammatory or potentiate endogenous anti-inflammatory mechanisms. Alternatively, a direct interaction of the GR complex with transcription factors may also be an important determinant of steroid action and a key mechanism by which glucocorticoids exert some anti-inflammatory activity.[1]

Abbreviations used

BDP:	Beclomethasone dipropionate
17-BMP:	Beclomethasone-17-monopropionate
FP:	Fluticasone propionate
GR:	Glucocorticoid receptor
GRE:	Glucocorticoid-responsive element
RBA:	Relative receptor binding affinity

The early development of corticosteroids based on the structure of cortisol focused on increasing topical potency and improving glucocorticoid selectivity. The first structure-activity studies attempted to find compounds with greater anti-inflammatory activity. This was achieved either by the insertion of an additional double bond at the 1,2 position in the steroid nucleus; by the introduction of 6α-fluoro, 6α-methyl, or 9α-fluoro substituents; or by a combination of these changes (Fig. 1). Although anti-inflammatory potency was potentiated, mineralocorticoid activity was increased to an even greater extent.[2] This effect was counteracted by further substitutions with α-hydroxyl, α-methyl, or β-methyl at the 16 position, for example, in dexamethasone (Fig. 1). A novel finding was that an ester function at the 16α, 17α, or 21α hydroxyl group was preferred, and this gave rise to betamethasone 17-valerate, triamcinolone 16,17-acetonide, and beclomethasone-17,21-dipropionate.[2] These compounds have proved to be of value in the treatment of the inflammatory component of bronchial asthma and rhinitis and have shown little detectable systemic activity when delivered by the topical route. However, concern that long-term therapy may result in a wide range of unacceptable systemic side effects such as adrenal suppression, bone fracture, osteoporosis, and inhibition of growth in children highlighted the need for steroids with a better therapeutic index.

DEVELOPMENT OF FLUTICASONE PROPIONATE

The development of fluticasone propionate was an attempt to produce a potent corticosteroid that exhibited improved airway selectivity (Table I) compared with earlier compounds. Lipophilicity was identified as an important physicochemical property for increased uptake and retention in lung tissue, resulting in enhanced lung-systemic distribution and greater affinity for the

From International Medical Affairs, Respiratory, Glaxo Wellcome Research and Development, Middlesex, United Kingdom.
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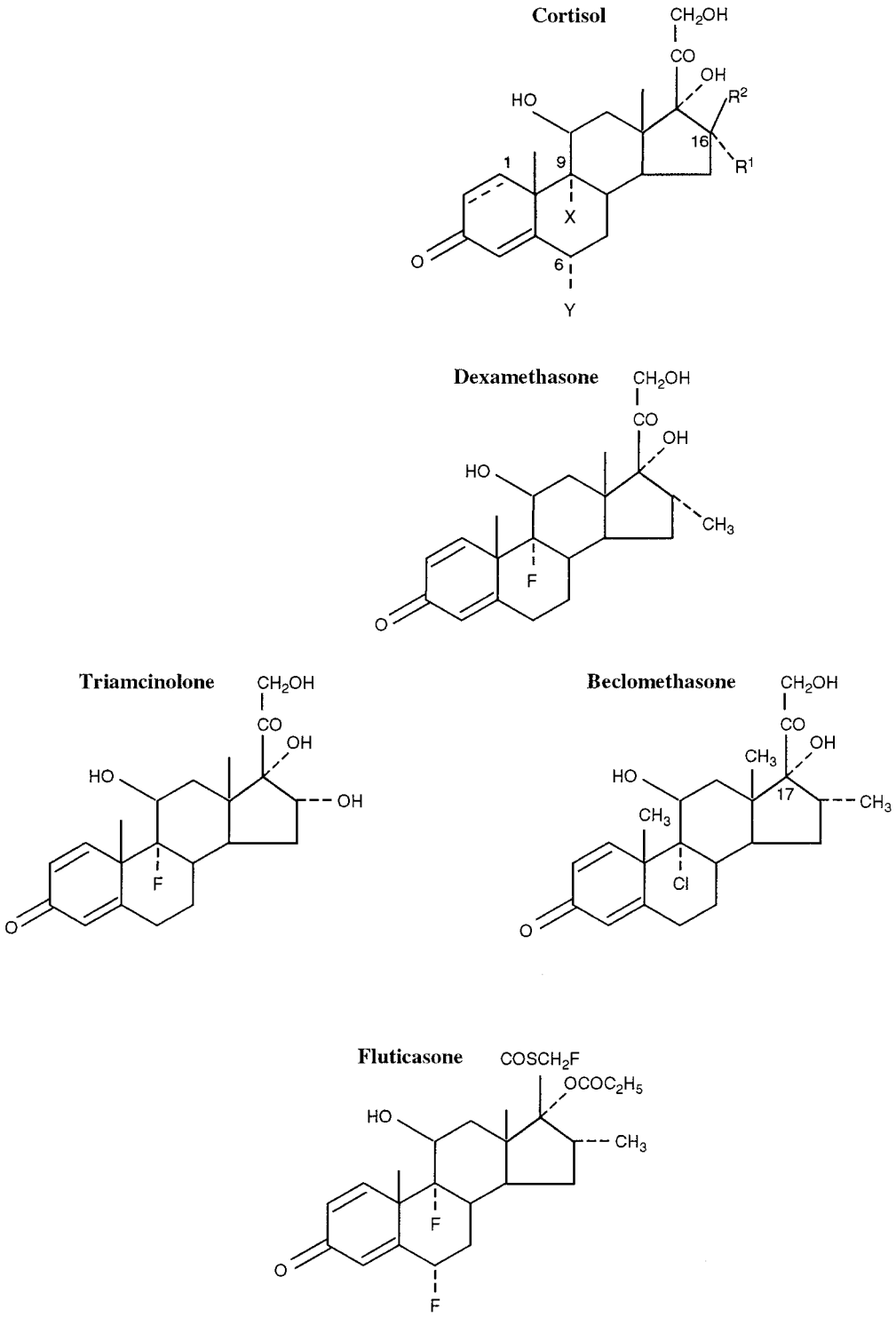

FIG. 1. Structural modifications of cortisol that produced the corticosteroids: dexamethasone, triamcinolone acetonide, beclomethasone dipropionate, and fluticasone propionate.

GR. The androstane nucleus, which is highly lipophilic, was therefore selected as the basis of the chemical program.[3] Topical activity was assessed by inhibition of croton oil–induced inflammation of the ear in a mouse model[4] and inhibitory activity at the hypothalamic-pituitary-adrenal (HPA) axis assessed by measuring reductions in circulating corticosteroids in response to ether stress.[5] The vasoconstriction/skin blanching assay[6]

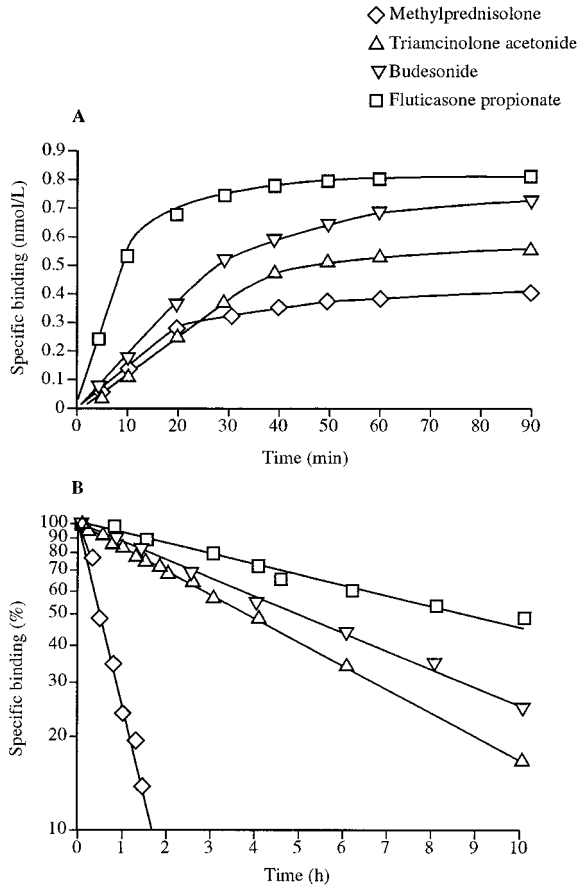

FIG. 2. Kinetics of **(A)** association and **(B)** dissociation of methylprednisolone, triamcinolone acetonide, budesonide, and fluticasone propionate with the glucocorticoid receptor in human lung tissue. Data from references 10 and 14.

was then used to confirm activity in human beings and to rank compounds in order of anti-inflammatory potency.

The androstane 17β-carboxylates, which lack the normal two-carbon side-chain of anti-inflammatory corticosteroids at the 17 position, were of particular interest.[3] The 17α-hydroxyl,17β-carboxylic acid was without activity in the vasoconstriction assay, with esterification being necessary for topical activity. Enzymatic hydrolysis of either ester function, which can occur in vivo, would therefore lead to inactive metabolites. The 17β-carboxylate series was superseded by the corresponding 17β-carbothioates.[3] Fluoromethyl analogues were, in general, more active than the corresponding chloromethyl compounds, with the 17-propionate being preferred over the acetate or butyrate; in addition, the presence of an α-CH_3 at position 16 reduced HPA axis–suppressing activity (Table II). The most active compound in the anti-inflammatory and vasoconstriction tests was the 6α,9α-difluoro, 17α-propionyl, 17β-carbothioate (fluticasone propionate), which was approximately 2-fold and 10-fold more potent than BDP and fluocinolone acetonide, respectively (Table II). Its low activity in inhibiting HPA axis function resulted from FP undergoing complete first-pass metabolism in the liver to the inactive 17β-carboxylic acid. X-ray crystallography has shown that the carbonyl of the 17β-substituent lies below the plane of the ring rather than above it, which is observed for other anti-inflammatory steroids.[7] This unusual shape, in which the carbothioate ester has increased accessibility, may explain why FP readily undergoes enzymatic hydrolysis. FP therefore has a high calculated therapeutic index (anti-inflammatory potency/HPA inhibitory potency) of 91, compared with 0.4 and 1.0 for BDP and fluocinolone acetonide, respectively.[8]

TABLE I. Criteria for improved airway selectivity of corticosteroids

Pharmacodynamics
• High glucocorticoid receptor affinity
• Optimal glucocorticoid receptor kinetics
• High intrinsic steroid potency/high topical anti-inflammatory activity
• High glucocorticoid receptor specificity
Pharmacokinetics
• Low oral bioavailability
• Increased uptake/retention in lung tissue
• Rapid systemic clearance
• Extrapulmonary metabolism to inactive metabolite(s)
• High lung:systemic distribution ratio

FP is 3 and 300 times more lipophilic than BDP and budesonide, respectively, and >1000-fold more lipophilic than either flunisolide or triamcinolone acetonide.[9] This degree of lipophilicity gives FP increased deposition in lung tissue and a slower release from the lung lipid compartment. In human lung fragments and nasal tissue in vitro, uptake and retention of corticosteroids is in the rank order FP > BDP > 17-BMP > budesonide > flunisolide > hydrocortisone.[10, 11] In patients with asthma, after inhalation of a 1 mg dose, FP exhibits a lung:systemic distribution ratio of 70 to 100,[12] compared with previous reports of 7 to 10 for budesonide.[13]

RECEPTOR PHARMACOLOGY

FP has a high affinity for the human lung GR (0.5 nmol/L),[14] which is 1.5-fold higher than 17-BMP and mometasone furoate, 3-fold higher than budesonide, and 10-fold higher than triamcinolone acetonide and flunisolide (Table III). Unlike budesonide, which is a racemic mixture of 22R and 22S enantiomers, FP does not have a chiral center and therefore the measured affinity represents the affinity of the molecule and not the contribution of the individual enantiomers. In contrast to 17-BMP, the metabolite of BDP that has a relative receptor binding affinity (RBA) 5-fold higher than the parent molecule, budesonide, with an RBA of 7.8, undergoes a marked reduction in activity when metabolized to either 6-hydroxy-budesonide (RBA = 0.06) or 16-α-hydroxy-prednisolone (RBA = 0.03). The

TABLE II. Structure-activity of halomethyl-androstane-17β-carbothioate analogues

Z	Y	X	R	16	Topical anti-inflammatory activity*	HPA suppression†	Cutaneous vasoconstriction‡
F	H	CI	C_2H_5	H	20	100	916
F	H	F	C_2H_5	H	63	149	1984
F	F	CI	C_2H_5	αCH_3	56	0.04	124
F§	F	F	C_2H_5	αCH_3	113	1.0	945
F	F	F	CH_3	αCH_3	76	2.9	392
F	F	F	C_3H_7	αCH_3	55	0.7	299
F	F	F	C_2H_5	βCH_3	197	>100	1048

Results are expressed relative to fluocinolone acetonide as standard (100). Data from Reference 3.
*Assessed with the croton oil ear assay in mice.[4]
†Assessed with the ether stress assay in rodents.[5]
‡Assessed with the skin blanching test in human volunteers.[6]
§Structure of fluticasone propionate.

TABLE III. Comparison of corticosteroid-glucocorticoid receptor affinity in human lung and potency in the cutaneous vasoconstriction test

Corticosteroid	Relative glucocorticoid receptor affinity*	Relative vasoconstrictor activity†
Fluocinolone acetonide	1.0	1.0
Beclomethasone-17-monopropionate	3.3	2.0
Triamcinolone acetonide	0.5	0.4
Flunisolide	0.45	0.5
Mometasone furoate	3.3	3.0
Budesonide	2.5	1.5
Fluticasone propionate	5.0	5.0

Activities are quoted relative to fluocinolone acetonide as standard (1.0).
*Data from Reference 14.
†Data from Reference 6.

TABLE IV. Corticosteroid-induced inhibition of human inflammatory cells

Corticosteroid	IC_{50} (nmol/L)			
	T-cell IL-5 release*	T-cell proliferation†	Basophil histamine release‡	Eosinophil apoptosis§
Beclomethasone dipropionate	7.7	10.0	1.0	138.7
Triamcinolone acetonide	9.8	1.0	20.0	23.8
Budesonide	1.7	0.2	0.6	8.5
Mometasone furoate	0.3	. . .	0.3	. . .
Fluticasone propionate	0.2	0.05	0.03	1.7

*Data from Reference 19.
†Data from Reference 18.
‡Data from Reference 20.
§Data from Reference 21.

17β-carboxylic acid metabolite of FP has negligible pharmacologic activity, with an RBA <0.01 at the GR.[9] The rate of association of steroid with the cytosolic GR is fastest for FP, followed by budesonide, triamcinolone acetonide, and methyl prednisolone (Fig. 2). In contrast, the rate of dissociation of FP from the receptor complex is slower than that of budesonide, triamcinolone acetonide, dexamethasone, and methyl prednisolone (Fig. 2). These differences in GR kinetics for FP result in differences in the stability of the steroid-receptor complex, which mediates the biologic and therapeutic activity of glucocorticoids.[1] The half-life of the steroid-receptor complex for FP is >10 hours, compared with approximately 3.5, 4.0, 5.0, and 7.5 hours for flunisolide, triamcinolone acetonide, budesonide, and 17-BMP, respectively.[9] FP is highly selective for the GR with <0.001 of the relative potency at human androgen, estrogen, and mineralocorticoid receptors.[15] The selectivity ratio of FP for the GR over the progestagen receptor is 1430, compared with 267 and 237 for 17-BMP and budesonide, respectively.

ANTI-INFLAMMATORY ACTIVITY

The steroid receptor profile of FP imparts a high topical anti-inflammatory activity. The active FP-GR complex binds to the GRE on target genes (EC_{50} = 3 nmol/L) or interacts directly with activating protein-1 and/or nuclear factor-kB transcription factors (EC_{50} range 0.01 to 0.1 nmol/L) at significantly lower concentrations than either dexamethasone or budesonide.[16] This has a good correlation with the respective potency of FP in inhibiting GRE-dependent cytokine (IL-6, IL-8) synthesis (IC_{50} range 5 to 10 nmol/L) and non–GRE-dependent cytokines such as tumor necrosis factor-α (TNFα) and granulocyte-macrophage colony stimulating factor (IC_{50} range 0.01 to 0.1 nmol/L).

There is also a good correlation between the relative affinity of these corticosteroids for the GR and their relative potency in a number of intact inflammatory cell systems (Table IV). For example, FP is more potent than dexamethasone, BDP, and budesonide in inhibiting human T-cell migration[17] and proliferation,[18] with IC_{50}

values of 0.3, 5.9, 2.0, and 0.8 nmol/L. Similarly, anti–CD3/CD28-induced IL-5 and IL-4 secretion from CD4+ T cells is inhibited by corticosteroids, with a rank order of potency of FP > mometasone furoate > budesonide > BDP > triamcinolone acetonide.[19] FP inhibits anti–IgE-stimulated histamine release from human basophils with an IC_{50} of 0.03 nmol/L, compared with 0.3, 0.6, 1, and 20 nmol/L for mometasone furoate, budesonide, BDP, and triamcinolone acetonide, respectively.[20] Corticosteroids, in the presence of IL-5, induce concentration-dependent apoptosis of eosinophils, with FP (EC_{50} = 1.7 nmol/L) being 5 times more potent than budesonide and approximately 10 times more potent than triamcinolone acetonide and flunisolide.[21] FP is also potent in inhibiting cytokine-induced adhesion molecule expression. At 1 nmol/L, FP inhibits TNFα-stimulated E-selectin in human endothelial cells,[22] whereas 8-fold higher concentrations of budesonide are required for the same effect. At a concentration of 100 nmol/L, FP is more effective than budesonide or triamcinolone acetonide in inhibiting intracellular adhesion molecule-1 expression in airway epithelial cells.[23] Finally, Abbin ante-Nissen et al.[24] have shown that corticosteroids induce the synthesis of the antiprotease, secretory leukocyte protease inhibitor (SLPI), in human airway epithelial cells. FP is the most potent steroid in inducing SLPI, with an EC_{50} of 0.1 nmol/L compared with 1, 5, and 2 nmol/L for triamcinolone acetonide, methylprednisolone, and dexamethasone, respectively.

The rank order of affinity of corticosteroids at the GR and their anti-inflammatory potency in vivo are similar. In the McKenzie test, in which the cutaneous vasoconstrictor and skin blanching response is used to rank anti-inflammatory potency of topical corticosteroids,[6] FP is 1.5-, 2.5-, and 3-fold more potent than 17-BMP, mometasone furoate, and budesonide, respectively, and 10-fold more potent than triamcinolone acetonide and flunisolide (Table III). This is in agreement with Dahlberg et al.,[25] who had previously reported that the RBA predicts relative potency for inhibition of edema.

CLINICAL STUDIES

In patients with asthma, FP treatment (1 mg twice daily for 2 months) significantly reduced the numbers of mast cells (by 80.2%), eosinophils (by 93.6%), and T cells (CD3, CD4, CD8, CD25; mean reduction of 86.5%) in bronchial biopsy specimens.[26] Similarly, the presence of dendritic (CD1a), IgE+, and HLA-DR+ cells in the lamina propria was decreased after FP 1 mg daily for 3 months,[27] suggesting attenuation of antigen recognition, processing, and presentation. Finally, FP (500 μg twice daily for 8 weeks) results in a marked decrease in the bronchoalveolar lavage levels of metalloprotease and an increase in the concentration of the endogenous tissue inhibitor of metalloproteases (TIMPS),[28] both of which have been implicated in matrix protein deposition and basement membrane thickening. FP, therefore, has good activity against the chronic inflammatory component of bronchial asthma and may attenuate the degree of airway remodeling.

The development of FP has resulted in a corticosteroid molecule with increased intrinsic glucocorticoid potency and potent anti-inflammatory activity, coupled with improved airway selectivity.[29] FP is of considerable clinical importance in the treatment of asthma and rhinitis.

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