

A novel pharmacologic action of glucocorticosteroids on leukotriene C₄ catabolism

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Leukotriene (LT) C₄, a potent chemical mediator in human bronchial asthma, is metabolized to less active LTE₄ via LTD₄ in 2 consecutive enzymatic reactions by γ -glutamyl transpeptidases and dipeptidases. We examined whether this inactivation process of LTC₄ was affected by fluticasone propionate, beclomethasone dipropionate, disodium cromoglycate, and salbutamol sulfate in transformed human bronchial epithelial cells. Fluticasone propionate and beclomethasone dipropionate accelerated LTC₄ catabolism by inducing activity of a LTC₄-degrading enzyme, γ -glutamyl transpeptidase-related enzyme (γ -GTPRE), in transformed human bronchial epithelial cells. The activation of γ -GTPRE might be regulated transcriptionally. This is a novel regulatory mechanism by which glucocorticosteroids exert antiasthma activities. (*J Allergy Clin Immunol* 2001;108:122-4.)

Key words: Beclomethasone dipropionate, cysteinyl leukotrienes, fluticasone propionate, γ -glutamyl transpeptidase, γ -glutamyl transpeptidase-related enzyme, human bronchial epithelial cells

Cysteinyl leukotrienes (cysLTs)—LTC₄, LTD₄, and LTE₄—play important roles in the pathogenesis of bronchial asthma. Clinical effectiveness of cysLT-receptor antagonists, which were recently developed and which have recently been used in patients, supports this hypothesis.¹ Of these cysLTs, LTC₄ and LTD₄ are biologically more active than LTE₄. LTC₄ is enzymatically metabolized to LTE₄ via LTD₄ by 2 consecutive LTC₄ catabolic enzymes, γ -glutamyl transpeptidase (γ -GTP) and dipeptidase. Recently, another γ -GTP-like activity was identified and cloned in human beings; it has been named γ -GTP-related enzyme (γ -GTPRE).²

Enzymatic transformation of LTC₄ to less active LTE₄ via LTD₄ by these LTC₄ catabolic enzymes might be an important consideration in the treatment of asthma. We have investigated whether glucocorticosteroids (GCSs) and other antiasthma drugs affect the catabolic pathway of LTC₄ to LTE₄ in transformed human bronchial epithelial cell line (16HBE) cells. We have also tried to determine whether GCS treatment influences mRNA expression of γ -GTP and γ -GTPRE.

Abbreviations used

16HBE:	Transformed human bronchial epithelial cell line
BDP:	Beclomethasone dipropionate
BMP:	Beclomethasone monopropionate
CysLT:	Cysteinyl leukotriene
DSCG:	Disodium cromoglycate
FP:	Fluticasone propionate
γ -GTP:	γ -glutamyl transpeptidase
γ -GTPRE:	γ -glutamyl transpeptidase-related enzyme
GCS:	Glucocorticosteroid
LT:	Leukotriene

METHODS

Transformed human bronchial epithelial cells that retain differentiated morphology and function of normal human airway epithelium³ were cultured in minimum essential medium supplemented with 10% heat-inactive FCS. The cells were incubated with fluticasone propionate (FP) at 10⁻⁸ mol/L, beclomethasone dipropionate (BDP) at 10⁻⁸ mol/L, disodium cromoglycate (DSCG) at 10⁻⁵ mol/L, or salbutamol sulfate at 10⁻⁵ mol/L for 2 days.

The cells were harvested and washed with PBS; cell lysates were then prepared as the enzyme source for enzyme assays. The lysates were incubated with authentic LTC₄ for 60 minutes. The enzymatic transformation from LTC₄ to LTD₄ and LTE₄ was assayed by means of HPLC after the samples were semipurified and concentrated according to procedures previously reported.⁴ The γ -GTP activity was determined by rate-kinetic assay through use of L- γ -glutamyl-p-nitroanilide and glycylglycine as the substrate.

After total RNA was extracted from the cells, complementary DNA was synthesized by RT reaction; PCR was then carried out through use of the following primers: γ -GTP, 5'-CCAGGACG-GCCAGGTCCG, 5'-CGATGAAGGTGGACGCGAT; γ -GTPRE, 5'-ACAGGGAAGGTGGAGGTCATC, 5'-GTCTCTACAAGGTG-GTGGTAC; beta-actin, 5'-TCCTGTGGCATCACGAAACT, 5'-TAGCAGGTGGCGTTTACGAAG. The PCR products were electrophoresed in 2.5% agarose gel and then stained with ethidium bromide. mRNA abundance was visualized under UV exposure and photographed through use of an image analyzer (Image Master VDS; Pharmacia Biotech, Tokyo, Japan). The levels of PCR products were analyzed through use of the NIH image program and compared with that of β -actin PCR product as an internal control.

To assess the BDP hydrolysis in 16HBE cells, we measured the levels of BDP and the metabolites 17-beclomethasone monopropionate (BMP) and 21-BMP. In brief, nontreated 16HBE cells were incubated with 100 μ g/mL of BDP for 0, 3, 6, and 24 hours in minimum essential medium with 10% heat-inactive FBS. The media were collected and applied to HPLC as samples. The samples were delivered with acetonitrile, methanol, water, acetic acid (10:280:170:1 [vol/vol]) at 1.2 mL/min, and the absorbance was monitored by means of a spectrophotometer at 242 nm.

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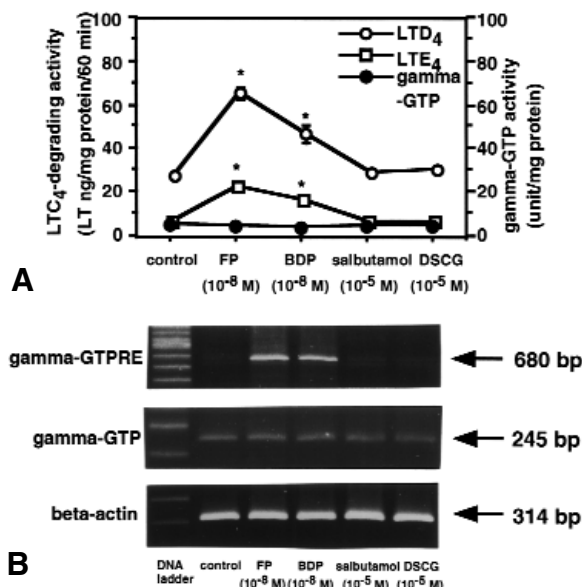


FIG 1. A, After pretreatment with various antiasthma drugs for 2 days, cell lysate was prepared and incubated with authentic LTC₄ for 60 minutes at 37°C. The enzymatic conversion of LTC₄ to LTD₄ (open circle) and LTE₄ (open square) was measured by means of HPLC. Enzyme activity of γ -GTP (solid circle) was determined by rate-kinetic assay through use of L- γ -glutamyl-p-nitroanilide and glycylglycine as the substrate. Data are given as means $\pm$ SEMs (n = 4). Asterisk indicates P value of <.05 versus control. **B**, Messenger RNA expression for γ -GTPRE and γ -GTP was examined by means of RT-PCR after cells were treated as described above. FP, Fluticasone propionate; BDP, beclomethasone dipropionate; salbutamol, salbutamol sulfate; DSCG, disodium cromoglycate.

Statistical analysis was performed through use of 1-way analysis of variance. A P value of <.05 was defined as statistically significant.

RESULTS

As shown in Fig 1, A, the LTC₄ catabolism values after incubation with FP and BDP were 64.8 ± 2.9 and 46.0 ± 4.1 (LTD₄ ng/mg protein/60 min), respectively, which were significantly higher than the value for the control, 26.5 ± 1.0 (LTD₄ ng/mg protein/60 min). The final conversion to LTE₄ was also accelerated by FP and BDP. Activity of γ -GTP, determined by means of rate-kinetic assay through use of L- γ -glutamyl-p-nitroanilide and glycylglycine, was not increased by either FP or BDP. The abundance of mRNA for γ -GTPRE was enhanced by the treatment with FP or BDP, but that for γ -GTP was not changed (Fig 1, B). DSCG and salbutamol sulfate showed no significant effects on the catabolic process of LTC₄ to LTE₄.

To confirm the BDP hydrolysis in 16HBE cells, we measured the levels of BDP and the metabolites 17-BMP and 21-BMP in medium after incubation with 16HBE cells and BDP. The peaks of authentic 21-BMP, 17-BMP, and BDP are shown in Fig 2, A, the retention times of which were approximately 8, 10, and 30 minutes, respectively. The peak of 17-BMP appeared and increased time-dependently after incubation with 16HBE cells and BDP (Fig 2, B, D, and E). These results indicate that BDP was converted to 17-BMP, an active form of BDP, in 16HBE cells.

DISCUSSION

The data indicated that FP and BDP accelerate the conversion of LTC₄ to LTD₄ and finally lead to the accel-

erated degradation LTC₄ to less active LTE₄ via LTD₄. This action was observed only after cells were exposed to FP and BDP for more than 2 days, suggesting new protein synthesis. Results of RT-PCR suggest that this acceleration was mediated by transcriptional up-regulation of γ -GTPRE but not γ -GTP. BDP, an inactive prodrug that does not bind to the glucocorticoid receptor, exerts pharmacologic action after being hydrolyzed in human lung to an active metabolite, 17-BMP.⁵ We confirmed that BDP was converted to 17-BMP in 16HBE cells.

Enzymatic conversion of LTC₄ to LTD₄ had been considered to be exerted by γ -GTP. However, another γ -GTP-like activity (γ -GTPRE) was recently identified and cloned.² γ -GTPRE shares an overall 40% amino acid sequence identity with γ -GTP and is capable of cleaving the γ -glutamyl linkage of LTC₄ to form LTD₄. The results that FP and BDP had no significant effect on the enzyme activity of dipeptidase, which converts LTD₄ to LTE₄ (data not shown), also support our hypothesis.

Our results strongly suggest up-regulation of γ -GTPRE by FP and BDP in human bronchial epithelial cells. We speculate that regular use of FP and BDP induces new enzyme activity of γ -GTPRE in airways, up-regulates the catabolic process of LTC₄ to less active LTE₄, and therefore contributes to the antiasthma actions. Treatment of asthma with inhaled GCS is the gold standard for adult patients and even for children in severe cases.⁶ FP and BDP, the most widely marketed inhaled GCSs, inhibit the production of allergy-related, T_H2-type inflammatory cytokines, such as IL-4, IL-5, and IL-13, by transcriptional mechanisms. Although the inhibition of T_H2 cytokine production by GCSs is a gen-

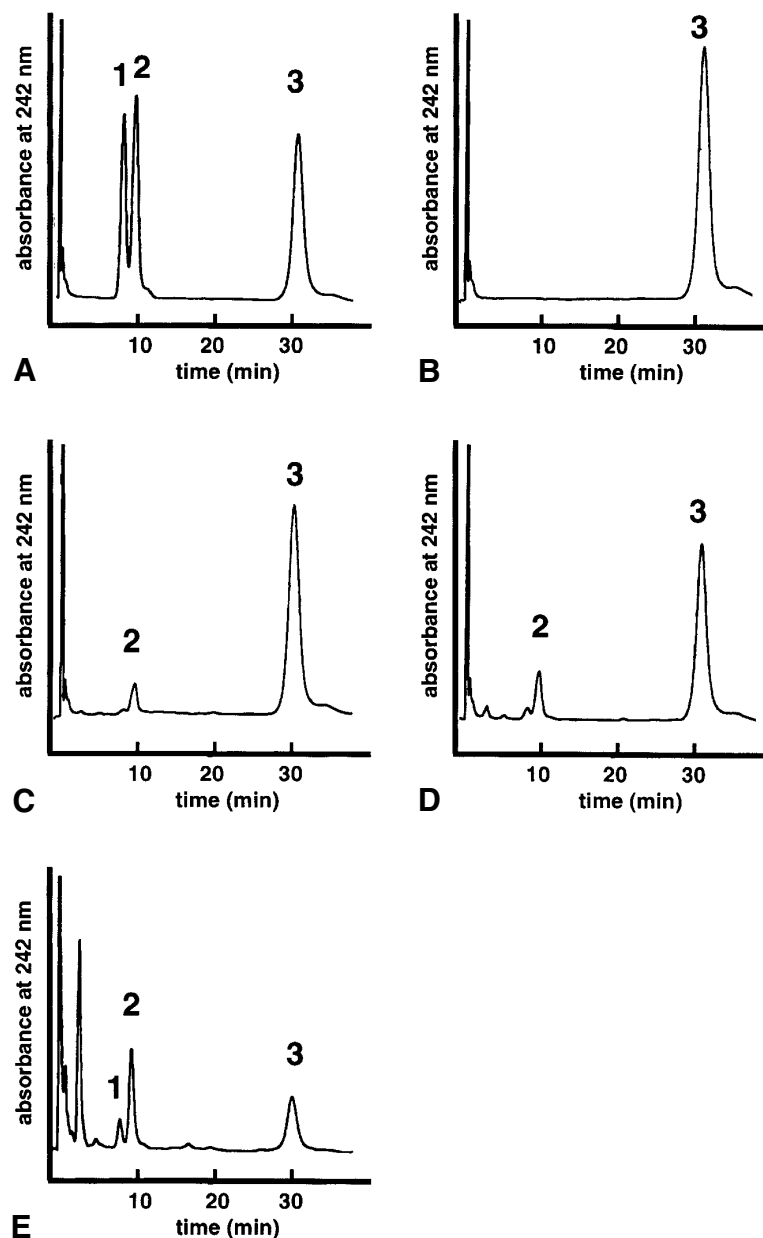


FIG 2. Levels of 21-BMP, 17-BMP, and BDP in incubation medium were measured by means of HPLC according to conditions described in Methods section. Peaks of authentic 21-BMP, 17-BMP, and BDP are shown (A). Retention times were approximately 8, 10, and 30 minutes (label 1, 21-BMP; label 2, 17-BMP; label 3, BDP), respectively. Authentic BDP was incubated with 16HBE cells for 0 hours (B), 3 hours (C), 6 hours (D), and 24 hours (E).

erally accepted mechanism in the treatment of asthma, the accelerated degradation of LTC_4 by inducing activity of γ -GTPRE with FP and BDP is a novel regulatory mechanism by which GCSs exert antiasthma activities.

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