Metabolism Kinetics of Beclomethasone Propionate Esters in Human Lung Homogenates

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Purpose. The purposes of this study were to characterize the kinetics of beclomethasone dipropionate (BDP) and its 17-monopropionate ester (17-BMP) in human lung 1000*g* supernatant (HLu) at 37°C, and to analyze the interindividual variability in the metabolism of BDP in HLu.

Methods. The concentrations of BDP and its metabolites were determined by HPLC with UV detection at 242 nm. Kinetics of BDP and 17-BMP decomposition were characterized by least-squares fitting of rate equations.

Results. The active metabolite 17-BMP was rapidly formed following the incubation of BDP in HLu. Kinetics of BDP and 17-BMP in HLu were nonlinear owing to product inhibition and enzyme saturation. A model taking into account the product inhibition provides a kinetic basis for understanding the *in vivo* behavior of BDP and its metabolites in human lung. There was approximately a 3.5-fold difference in the initial half-life of BDP in HLu observed in seven subjects.

Conclusions. An effective activation of BDP was demonstrated in HLu through the rapid formation of 17-BMP. Kinetics of BDP and 17-BMP in HLu were well characterized by the nonlinear kinetic model. Interindividual difference in the initial half-life of BDP was due mainly to esterase metabolizing activity rather than binding affinity.

KEY WORDS: metabolism; beclomethasone dipropionate; beclomethasone 17-monopropionate; nonlinear; kinetic model; human lung.

INTRODUCTION

Beclomethasone dipropionate (BDP) is an important glucocorticoid diester in the inhalation therapy of lung diseases such as asthma (1,2). It has been reported (3–5) that human lung tissue metabolizes BDP to beclomethasone 17-monopropionate (17-BMP) and beclomethasone (BOH) (Fig. 1). The relative binding affinities of 17-BMP and BOH for cytoplasmic glucocorticoid receptor of human lung are approximately 30 and 2 times greater than that of BDP, respectively (5,6).

The therapeutic index of BDP might be affected by the relative stability and intrinsic glucocorticoid activity of its degradation products. It has been suggested that the active metabolite 17-BMP which is formed in the lung may be rapidly absorbed into the systemic circulation (7–9), giving rise to concerns over the potential adverse systemic effects. Thus, the characterization of the kinetics of metabolism of BDP and 17-BMP in human lung is of considerable importance to the understanding of factors determining the ratio of local anti-inflammatory action to systemic activity.

Predictive models for the determination of *in vivo* metabolizing activity from *in vitro* data in humans have been attracting increased attention in recent years (10,11). Kinetic studies conducted over a range of concentrations may allow evaluation of the kinetics mechanistically and hence to predict the kinetic behavior of BDP and 17-BMP *in vivo* in the lung.

The present study reports the evaluation of kinetics and the development of a model for metabolism of BDP and 17-BMP in human lung 1000*g* supernatant (HLu) at 37°C. A preliminary analysis of the quantitative differences in the kinetics of BDP between individual samples of HLu is discussed.

MATERIALS AND METHODS

Materials

BDP, BOH, dexamethasone 21-acetate, sucrose, total protein reagent, protein standard solution and dichloromethane (99.9%, HPLC grade) were purchased from Sigma Chemical Co. (St. Louis, MO). Pure reference standards of BDP, 17-BMP and BOH were kindly provided by Glaxo Australia (Boronia, VIC). Methanol and acetonitrile were of ChromAR HPLC grade, sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, acetic acid and ethanol were of analytical reagent grade and obtained from commercial sources.

Preparation of Human Lung Supernatant

Human lung samples were obtained immediately after lung resection surgery from 12 subjects at three hospitals (Sydney, NSW, Australia). Written informed consent was obtained from each patient after full explanation of the purpose and risks of the procedures performed. The investigational protocol for all procedures was approved by the institutional Human Ethics Committee. The small pieces of lung tissue were obtained from upper, middle and lower lobes. They were homogenized (Kinematica Polytron) in 2.5 to 5 volumes of ice-cold isotonic 0.067 M sodium phosphate buffer (pH 7.4) containing 0.15 M sucrose for 3 × 10 sec. All preparations were carried out below 4°C. A separate HLu was prepared from tissue from each subject, on the day of tissue collection.

The HLu was obtained following centrifugation of the lung homogenate at 1000 *g* (4°C) for 10 min. HLu contains 5 major cell types namely endothelial cells, pulmonary macrophages, clara cells, type I and type II pneumocytes. Total protein concentration in HLu was determined spectrophotometrically at 540 nm (12), prior to storage at −80°C for no longer than one week. HLu was diluted with ice-cold homogenizing buffer, yielding a lung protein concentration of 4 mg/ml prior to incubation study. Demographic characteristics of subjects and the drugs used for kinetic study *in vitro* are given in Table I. Owing

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ABBREVIATIONS: AUC, Area under the concentration-time curve; BDP, Beclomethasone dipropionate; 17-BMP, Beclomethasone 17-monopropionate; BOH, Beclomethasone; HLu, Human lung 1000*g* supernatant; IS, Internal standard.

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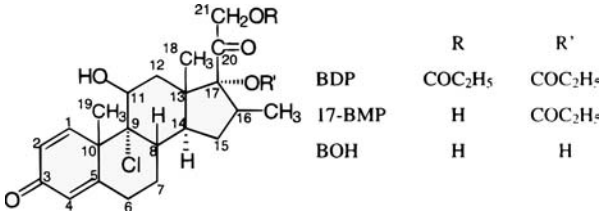

Fig. 1. Structural formulae of BDP, 17-BMP, and BOH.

to the limited volume of HLu obtained from subjects no. 8–12, kinetic studies using BDP as parent drug were only performed in HLu samples no. 1–7.

Kinetic Studies

All incubations were carried out in borosilicate test tubes under atmospheric conditions with gentle shaking at 37.0 ± 0.1°C in a water bath shielded from light. Prior to commencement, media were preadjusted, for 10 min, to the temperature of study. Kinetic studies were initiated by the addition of an ethanolic solution of parent drug to HLu yielding an initial concentration (C_o) in the range 10 to 40 μg/ml in ethanol/water (1:99, v/v), with the final lung protein concentration of 3.96 mg/ml. At predetermined time intervals, 0.5 ml samples were removed and immediately frozen on dry ice/ethanol. The samples were extracted and analyzed on the same day as the incubation performed. The concentrations of undegraded parent drug and its degradation products were determined by HPLC.

Extraction Procedure

Samples (0.5 ml) were spiked with 0.5 ml of a 40 μg/ml ethanolic solution of dexamethasone 21-acetate (internal standard/IS) and then extracted with 4 ml dichloromethane for 30 min, using a roller mixer, followed by centrifugation at 2500 rpm (20°C) for 15 min. The dichloromethane layer was collected and evaporated to dryness under a stream of N_2 at 30°C. The resulting residue was reconstituted in 250 μl mobile phase, transferred to a polypropylene microcentrifuge tube (1.5 ml, eppendorf) and centrifuged at 15,000 rpm for 2 min prior to injection onto the HPLC column (injection volume, 50 μl).

Table I. Demographic Data of the Subjects and Drugs Used for *In Vitro* Kinetic Study

Subject number	Age (yrs)	Sex	Smoking status	Disease type	Parent drug
1	15	Female	Unknown	PAS[a]	BDP, 17-BMP
2	75	Male	Smoker	Carcinoma	BDP, 17-BMP
3	68	Male	Nonsmoker	Carcinoma	BDP, 17-BMP
4	53	Male	Nonsmoker	Emphysema	BDP, 17-BMP
5	84	Male	Nonsmoker	Carcinoma	BDP, 17-BMP
6	65	Female	Smoker	Carcinoma	BDP, 17-BMP
7	73	Female	Nonsmoker	Carcinoma	BDP, 17-BMP
8	71	Male	Nonsmoker	Carcinoma	17-BMP
9	75	Male	Smoker	Carcinoma	17-BMP
10	70	Female	Nonsmoker	Carcinoma	17-BMP
11	81	Male	Smoker	Carcinoma	17-BMP
12	58	Male	Nonsmoker	Carcinoma	17-BMP

[a] PAS, pulmonary artery stenosis.

HPLC Analysis

Liquid chromatography was performed on an HPLC system consisting of a JASCO PU-980 solvent delivery system, a JASCO UV-975 UV/VIS detector (Tokyo, Japan) and an ICI AS 2000 automatic injector (Dandenong, VIC, Australia). The system was interfaced to a Delta 5.0 Chromatography data system from Digital Solutions (Margate, QLD, Australia). The HPLC column used was Alltima C_{18} (250 × 4.6 mm i.d., 5-μm particle size) from Alltech Associates (Baulkham Hills, NSW, Australia). The mobile phase was a mixture of methanol/water/acetonitrile/acetic acid (352:166:50:1, v/v), filtered through a Millipore 0.45 μm HVLP filter (Bedford, MA) and degassed by stirring under reduced pressure before use. HPLC was performed isocratically at ambient temperature and a flow rate of 1.3 ml/min with UV detection at 242 nm. Under this condition, retention times were 4.7 min for BOH, 6.0 min for IS, 9.0 min for 17-BMP and 20.0 min for BDP. HPLC linearity was determined for BDP, 17-BMP and BOH over the concentration range 0.1–50.0 μg/ml.

Kinetic Assessment

Initially kinetic studies using BDP alone as parent drug at a series of initial concentrations (10–40 μg/ml or 19–77 μM) in HLu were performed to analyze its kinetic behavior. Based on the metabolite profiles of BDP in HLu, a model was developed for decomposition of BDP, formation of 17-BMP and BOH, and subsequent decomposition of 17-BMP. To further test the model, studies of BDP kinetics were also conducted with the decomposition products either 17-BMP or BOH incorporated at time zero.

Data Analysis

The initial half-lives ($t_{1/2}$) were calculated from the apparent decomposition rate constants over the initial log-linear phase of decomposition (13). The area under the concentration-time curve (AUC value) was estimated by trapezoidal integrations (MOMENTS®). The nonlinear least-squares regression analysis for curve-fitting was performed using SCIENTIST® (Micromath Scientific Software, Salt Lake City, UT). The correlation (r^2) was used as an indicator of goodness-of-fit of the equation to the experimental data. Results were expressed as the mean ± S.D.

RESULTS AND DISCUSSION

Degradation Reactions

The reaction involved in the BDP decomposition in HLu at 37°C is ester hydrolysis, which is largely enzyme-catalyzed. Following the incubation of BDP in HLu, the active metabolite 17-BMP was rapidly formed and then slowly biotransformed to BOH. The rapid decomposition of BDP in HLu following the incubation of BDP alone (initial $t_{1/2}$ = 34.9 ± 15.2 min, n = 7) may be attributed to the relatively high esterase activity for BDP in human lung. The decomposition of 17-BMP in HLu was substantially slower (initial $t_{1/2}$ = 3.5 ± 1.2 h, n = 12)

than that of BDP. The ultimate product, BOH was found to be relatively stable in HLu. We speculate that most of the inhaled dose of BDP which reaches the lower respiratory tract is hydrolyzed to 17-BMP, prior to its association with the glucocorticoid receptor within target cells.

The plots of declining concentration of BDP after normalization with the initial concentration versus time following the incubation of BDP at a series of initial concentrations in HLu were not superimposable (Fig. 2). Thus BDP exhibited nonlinear kinetics in HLu over the concentration range examined. The normalized AUC values of BDP were also dependent on initial concentrations. BDP with a high initial concentration tended to decompose relatively slower than that with the lower one (Fig. 2).

One possible explanation of nonlinear kinetics is saturation of drug metabolizing enzymes. Thus the Michaelis-Menten kinetic model (Eq. 1) was fitted directly to the data obtained following the incubation of BDP over a range of concentrations (19 to 77 μM) in HLu:

$$-\frac{dC}{dt} = \frac{V_m C}{C + K_m} \quad (1)$$

where C is the concentration of BDP, dC/dt is the rate of change of C of BDP concentration at time t, V_m is the maximum dC/dt and K_m is the apparent Michaelis constant, that is the concentration of BDP reaching half of its maximum dC/dt. The kinetic profiles, especially for BDP concentration at the latter time points were not generally well characterized by Eq. 1. Thus the nonlinear kinetics of BDP in HLu could not solely be explained by enzyme saturation.

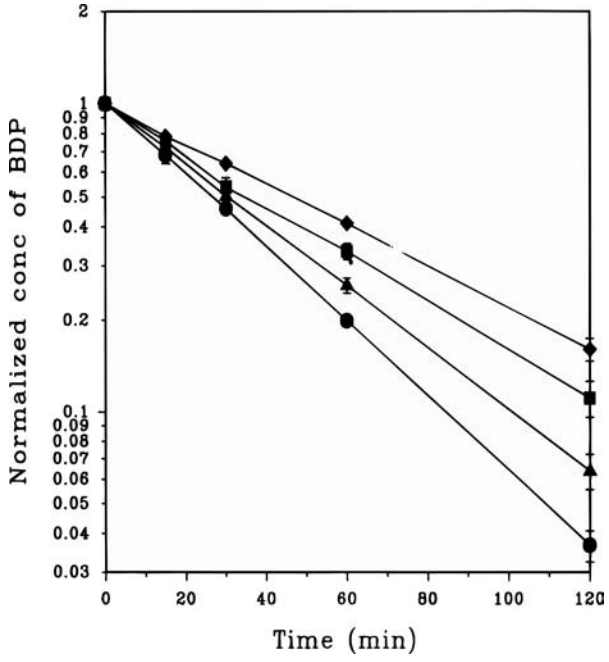

Fig. 2. Kinetic profiles of BDP normalized to the initial concentration (C_o) following incubation at various initial concentrations in HLu at 37°C: ♦, C_o = 40 μg/ml or 77 μM; ■, C_o = 30 μg/ml or 58 μM; ▲, C_o = 20 μg/ml or 38 μM; ●, C_o = 10 μg/ml or 19 μM. The vertical bars indicate the S.D. of five determinations. When no bars are shown, the S.D. fell within the symbol dimensions.

Table II. The Concentration-dependent Inhibition Effect of 17-BMP on the Kinetics of Its Parent, BDP (C_o = 10 μg/ml or 19 μM) in HLu at 37°C[a]

Kinetic parameters of BDP[b]	In the presence of 17-BMP at time zero		
	22 μM	54 μM	86 μM
Initial $t_{1/2}$ (min)	8.3 ± 0.1	9.6 ± 0.3	10.8 ± 0.2
$AUC_{0-\infty}$ (μM min)	254 ± 5	284 ± 5	324 ± 7

[a] Experimental data represent the mean ± S.D. of five determinations.
[b] Kinetic parameters among groups were significantly different ($p < 0.0005$) by single-factor ANOVA.

A kinetic model including product inhibition was proposed based on the evidence that the kinetics of the parent compound were altered when the decomposition products were added at time zero. Significant increases in the initial half-life and $AUC_{0-\infty}$ values of BDP following its incubation in HLu were observed, by increasing the concentration of 17-BMP (Table II). Similar trends were also demonstrated for the kinetics of parent 17-BMP in HLu when BOH was added at time zero (Table III). This might be attributed to the fact that BDP, 17-BMP and BOH are co-substrates for the same enzyme.

Development of a Nonlinear Kinetic Model with Product Inhibition

Based on the assumption of product inhibition and enzyme saturation, the following model is proposed to describe the nonlinear kinetics of metabolism of BDP in HLu:

$$\mathrm{BDP} + \mathrm{E} \overset{Ka_{BDP}}{\rightleftarrows} [\mathrm{E}\text{ - }\mathrm{BDP}] \overset{\lambda_{BDP}}{\rightarrow} \text{17-BMP}$$

$$+ \mathrm{E} \overset{Ka_{BMP}}{\rightleftarrows} [\mathrm{E}\text{ - 17-BMP}] \overset{\lambda_{BMP}}{\rightarrow} \mathrm{BOH} + \mathrm{E} \overset{Ka_{BOH}}{\rightleftarrows} [\mathrm{E} - \mathrm{BOH}]$$

where E is the enzyme (esterase), Ka is the equilibrium association constant for esterase-glucocorticoid complex and λ is the pseudo-first-order decomposition rate constant of the complex.

According to the model, BDP is first bound to the esterase enzyme, followed by decomposition to yield 17-BMP and the enzyme. Similarly 17-BMP binds to the esterase to form a complex which decomposes to release BOH. In the final step, BOH binds to the enzyme to form a complex which does not further decompose. Because the lung esterase concentration was not determined in this study, the esterase metabolizing

Table III. The Concentration-dependent Inhibition Effect of BOH on the Kinetics of Its Parent, 17-BMP (C_o = 30 μg/ml or 65 μM) in HLu at 37°C[a]

Kinetic parameters of 17-BMP[b]	In the presence of BOH at time zero			
	0 μM (control)	24 μM	61 μM	98 μM
Initial $t_{1/2}$ (h)	1.9 ± 0.1	2.8 ± 0.2	4.4 ± 0.2	5.9 ± 0.4
AUC_{0-7h} (μM h)	202 ± 4	242 ± 4	285 ± 5	315 ± 5

[a] Experimental data represent the mean ± S.D. of five determinations.
[b] Kinetic parameters among control and treatment groups were significantly different ($p < 0.0005$) by single-factor ANOVA.

activity was characterized by the product $\lambda[E]_o$, instead of individual λ and $[E]_o$ values.

For the case of incubation of BDP in HLu, the rate equations are as follows:

$$\frac{d[BDP]}{dt} = -\frac{\lambda_{BDP}Ka_{BDP}[E]_o[BDP]}{1 + Ka_{BDP}[BDP] + Ka_{BMP}[17 - BMP] + Ka_{BOH}[BOH]} \quad (2)$$

$$\frac{d[17 - BMP]}{dt} = \frac{\lambda_{BDP}Ka_{BDP}[E]_o[BDP]}{1 + Ka_{BDP}[BDP] + Ka_{BMP}[17 - BMP] + Ka_{BOH}[BOH]} - \frac{\lambda_{BMP}Ka_{BMP}[E]_o[17 - BMP]}{1 + Ka_{BDP}[BDP] + Ka_{BMP}[17 - BMP] + Ka_{BOH}[BOH]} \quad (3)$$

$$\frac{d[BOH]}{dt} = \frac{\lambda_{BMP}Ka_{BMP}[E]_o[17 - BMP]}{1 + Ka_{BDP}[BDP] + Ka_{BMP}[17 - BMP] + Ka_{BOH}[BOH]} \quad (4)$$

The initial estimation of model parameters was carried out by investigating the initial linear part of the declining curve of BDP, following incubation over a range of concentrations in HLu. At early times, the concentrations of products, namely 17-BMP and BOH, were relatively insignificant compared to that of BDP. Thus Eq. 2 can be expressed as:

$$\frac{d[BDP]}{dt} \approx -\frac{\lambda_{BDP}[E]_oKa_{BDP}}{1 + Ka_{BDP}[BDP]}[BDP] \quad (5)$$

When the natural logarithm of concentration of BDP is plotted against the time, the initial slope of the line is $\lambda_{BDP}[E]_o Ka_{BDP}/1 + Ka_{BDP}[BDP]$. Thus approximate values for Ka and $\lambda[E]_o$ for BDP can be estimated, following its incubation at a series of initial concentrations in HLu.

Similarly, a nonlinear kinetic model of 17-BMP in HLu was proposed:

$$17\text{-BMP} + E \overset{Ka_{BMP}}{\rightleftarrows} [E - 17\text{-BMP}] \overset{\lambda_{BMP}}{\rightarrow} BOH$$

$$+ E \overset{Ka_{BOH}}{\rightleftarrows} [E - BOH]$$

For the case of incubation of 17-BMP in HLu, the rate equations are as follows:

$$\frac{d[17 - BMP]}{dt} = -\frac{\lambda_{BMP}Ka_{BMP}[E]_o[17 - BMP]}{1 + Ka_{BMP}[17 - BMP] + Ka_{BOH}[BOH]} \quad (6)$$

$$\frac{d[BOH]}{dt} = \frac{\lambda_{BMP}Ka_{BMP}[E]_o[17 - BMP]}{1 + Ka_{BMP}[17 - BMP] + Ka_{BOH}[BOH]} \quad (7)$$

Testing of the Kinetic Model

The proposed model for metabolism of BDP and 17-BMP was first tested by fitting the kinetic patterns following the incubation of either BDP or 17-BMP alone at two different initial concentrations, namely 10 and 40 μg/ml in HLu. In the case of incubation of BDP, the concentration-time data for BDP and the degradation products 17-BMP and BOH were simultaneously fitted to the kinetic model described by Eqs. 2–4, with the zero time concentration of 17-BMP and BOH set to zero. In the case of incubation with 17-BMP, the concentration-time data for 17-BMP and the degradation product BOH were simultaneously fitted to the kinetic model described by Eqs. 6 and 7, with the zero time concentration of BOH set to zero.

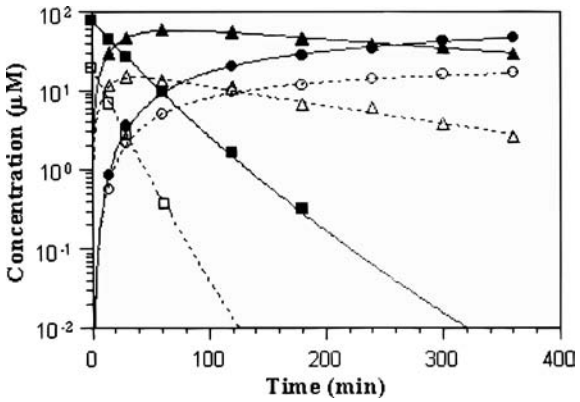

Fig. 3. Kinetic patterns following incubation of BDP at two different initial concentrations (C_o) in HLu at 37°C: C_o = 40 μg/ml or 77 μM (■, BDP; ▲, 17-BMP; ●, BOH); C_o = 10 μg/ml or 19 μM (□, BDP; △, 17-BMP; ○, BOH). Symbols are the mean value of five determinations and lines show least-squares fitting of the data to Eqs. 2–4.

Good correlations ($r^2 > 0.98$) between observed values and those fitted to Eqs. 2–4 were obtained, following the incubation of BDP at two different initial concentrations in HLu (Fig. 3). Likewise, the fit of Eqs. 6 and 7 to the experimental data obtained following the incubation of 17-BMP at two different initial concentrations in HLu was good ($r^2 > 0.98$). The alteration in the initial half-life of 17-BMP following its incubation at two different initial concentrations was not accompanied by significant changes in the model parameters namely Ka and $\lambda[E]_o$ for 17-BMP and Ka for BOH (Table IV). This is an indication that the model parameters were independent of initial concentrations.

The model was further tested by fitting the data obtained following the incubation of BDP in HLu with the products either 17-BMP or BOH added at time zero. The fitting procedure was the same as described above for incubation with BDP alone, except that at time zero 17-BMP and BOH concentrations were set at nonzero values corresponding to the experimental

Table IV. Kinetic Parameters of 17-BMP and BOH Following the Incubation of 17-BMP at Two Different Initial Concentrations (C_o) in HLu at 37°C[a]

Kinetic parameters	C_o = 40 μg/ml	C_o = 10 μg/ml	p value[b]
Initial $t_{1/2}$ (h)	2.30 ± 0.08	1.57 ± 0.02	<0.0005
Ka_{BMP} (μM^{-1})	0.009 ± 0.003	0.009 ± 0.002	>0.9
Ka_{BOH} (μM^{-1})	0.049 ± 0.003	0.050 ± 0.008	>0.8
$\lambda_{BMP}[E]_o$ (μM min^{-1})	1.0 ± 0.1	1.01 ± 0.05	>0.8

[a] Experimental data represent the mean ± S.D. of five determinations.
[b] Paired-sample t-test between two different initial concentration groups.

conditions. Good correlations ($r^2 > 0.98$) between experimental values and those fitted to Eqs. 2–4 were demonstrated. The model parameters namely Ka and λ $[E]_o$ for BDP and 17-BMP and Ka for BOH obtained following the incubation of BDP were unaffected by the inclusion of either 17-BMP or BOH at time zero (Table V). Overall, the proposed model can be applied following the incubation of either BDP alone or BDP with its products present at time zero.

In HLu, the initial half-life of 17-BMP (3.5 ± 1.2 h, n = 12) was approximately 6 times longer than that of BDP (0.6 ± 0.3 h, n = 7). This is in accordance with the lower esterase metabolizing activity for 17-BMP (0.6 ± 0.2 μM min^{-1}, n = 12) compared to that for BDP (2.8 ± 1.4 μM min^{-1}, n = 7). The respective binding affinities of glucocorticoids for lung esterase decreased in the following order: BOH (0.044 ± 0.009 μM^{-1}, n = 12) > BDP (0.026 ± 0.003 μM^{-1}, n = 7) > 17-BMP (0.015 ± 0.003 μM^{-1}, n = 12). Thus the relatively slow decomposition rate of the active metabolite 17-BMP in HLu may be associated with its low binding affinity for the lung esterase, the low esterase metabolizing activity for 17-BMP and the significant inhibition by the ultimate product BOH.

Interindividual Variability in Activation of BDP in HLu

There was an approximately 3.5-fold (CV = 43.6%, range 15.0–53.4 min) individual difference in the initial half-life of BDP in HLu, observed in seven subjects. Although both model parameters, namely Ka and $\lambda[E]_o$ differ significantly among individuals, the interindividual difference in the initial half-life of BDP was attributable overwhelmingly to variability in the term $\lambda[E]_o$ (CV = 49.6%, range 1.5–5.2 μM min^{-1}) rather than binding affinity for esterase (CV = 10.8%, range 0.023–0.031 μM^{-1}). The most likely explanation is variation in enzyme activity. However, the interindividual variability observed could also be partly due to experimental variability and inherent variations in the population sample (age, pathological conditions and cigarette smoking). The angiotensin I converting enzyme in rabbit lung develops with age *in utero* after birth (14,15). Destruction of alveoli in emphysema (16) results in the elimination of some metabolizing activity of type II pneumocytes (17). Smoking appears to be an effective enzyme inducer for benzo[a]pyrene hydroxylase activities in lung microsomes (18). In contrast, sex differences have no apparent effect on the pulmonary metabolism of benzo[a]pyrene by monooxygenases (19).

CONCLUSIONS

The rapid biotransformation of BDP to its active metabolite 17-BMP in HLu will favor a potent local anti-inflammatory action. The existence of product inhibition was demonstrated in the metabolism kinetics of BDP and 17-BMP in HLu. A nonlinear kinetic model capable of characterizing the activation of BDP to 17-BMP in HLu may be useful to predict the therapeutic efficacy following inhalation of BDP. The more rapid conversion of BDP to the more active 17-BMP which was observed in some individuals will promote a higher local anti-inflammatory effect in human lung.

APPENDIX

The following descriptions are aimed to show the derivations of nonlinear kinetic model of BDP in HLu, given by Eqs. 2–4.

$$\text{BDP} + \text{E} \overset{Ka_{BDP}}{\rightleftarrows} [\text{E-BDP}] \overset{\lambda_{BDP}}{\rightarrow} \text{17-BMP}$$

$$+ \text{E} \overset{Ka_{BMP}}{\rightleftarrows} [\text{E-17-BMP}] \overset{\lambda_{BMP}}{\rightarrow} \text{BOH} + \text{E} \overset{Ka_{BOH}}{\rightleftarrows} [\text{E-BOH}]$$

Competition occurring among these glucocorticoids for binding sites on the esterase is shown in the following equation:

$$[E]_o = [E] + Ka_{BDP}[E][BDP] + Ka_{BMP}[E][17\text{-BMP}] + Ka_{BOH}[E][BOH] \quad \text{(A1)}$$

where $[E]_o$ is the lung esterase concentration, [E] is the concentration of free esterase, and Ka_{BDP} [E] [BDP], Ka_{BMP} [E] [17-BMP] and Ka_{BOH} [E] [BOH] are the concentration of esterase which has been bound by BDP, 17-BMP and BOH, respectively. By rearrangement, Eq. A1 results in the following equation:

$$[E] = \frac{[E]_o}{1 + Ka_{BDP}[BDP] + Ka_{BMP}[17\text{-BMP}] + Ka_{BOH}[BOH]} \quad \text{(A2)}$$

The kinetic rate equations involved, following the incubation of BDP in HLu were as follows:

$$\frac{d[BDP]}{dt} = -\lambda_{BDP}Ka_{BDP}[E][BDP] \quad \text{(A3)}$$

which describes the rate of change of amount of BDP in HLu,

$$\frac{d[17-BMP]}{dt} = \lambda_{BDP}Ka_{BDP}[E][BDP] - \lambda_{BMP}Ka_{BMP}[E][17\text{-BMP}] \quad \text{(A4)}$$

which describes the rate of change of amount of 17-BMP in HLu, consisting of the rates of formation and decomposition of 17-BMP, and

Table V. Model Parameters of BDP, 17-BMP, and BOH Following the Incubation of BDP (C_o = 30 μg/ml or 58 μM) in HLu, Without (Control) and with the Decomposition Products Either 17-BMP or BOH Added at Time Zero, at a Concentration of 80 μM, at 37°C[a]

Model parameters	BDP only (control)	BDP + 17-BMP	BDP + BOH	p value[b]
Ka_{BDP} (μM^{-1})	0.025 ± 0.004	0.024 ± 0.002	0.026 ± 0.002	>0.6
Ka_{BMP} (μM^{-1})	0.017 ± 0.001	0.015 ± 0.002	0.016 ± 0.002	>0.1
Ka_{BOH} (μM^{-1})	0.044 ± 0.005	0.042 ± 0.002	0.045 ± 0.003	>0.3
λ_{BDP} $[E]_o$ (μM min^{-1})	4.2 ± 0.4	4.1 ± 0.4	4.0 ± 0.2	>0.7
λ_{BMP} $[E]_o$ (μM min^{-1})	0.76 ± 0.02	0.82 ± 0.06	0.80 ± 0.02	>0.05

[a] Experimental data represent the mean ± S.D. of five determinations.
[b] Single-factor ANOVA among control and treatment groups.

$$\frac{d[BOH]}{dt} = \lambda_{BMP} Ka_{BMP}[E][17\text{-BMP}] \qquad (A5)$$

which describes the rate of change of amount of BOH in HLu. Thus substitution of [E] from Eq. A2 into Eqs. A3–A5 will give Eqs. 2–4.

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