Comparative Kinetics of Metabolism of Beclomethasone Propionate Esters in Human Lung Homogenates and Plasma

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ABSTRACT: The systemic availability of inhaled beclomethasone dipropionate (BDP) is the net result of the absorption of the glucocorticoid from the lower respiratory and gastrointestinal tracts, and metabolism in the lung, plasma, and other sites. The metabolism kinetics of BDP and its active metabolite, beclomethasone 17-monopropionate (17-BMP), in human lung 1000 × *g* supernatant (HLu) and human plasma (HP) at 37 °C were compared. The effect of $MgCl_2$ and/or an NADPH-generating system on the decomposition of BDP and 17-BMP in HLu was also investigated. The concentrations of BDP and its metabolites were determined by HPLC with UV detection at 242 nm. Kinetics of decomposition of BDP and 17-BMP in HLu and HP were qualitatively and quantitatively different. The decomposition of BDP in HLu involved only hydrolysis. In comparison, three reactions are involved following incubation of BDP in HP; namely, hydrolysis, transesterification, and loss of hydrogen chloride. The hydrolysis of BDP and 17-BMP in HLu seem to be inhibited appreciably by $MgCl_2$ with the NADPH-generating system. Effective activation of BDP in HLu, in combination with transesterification of 17-BMP in HP, might favor a high ratio of local antiinflammatory activity to systemic side effects following inhalation of BDP. J Pharm Sci 89: 1143–1150, 2000

Keywords: kinetics; metabolism; beclomethasone dipropionate; lung; plasma

INTRODUCTION

Beclomethasone dipropionate (BDP) is a widely used glucocorticoid diester for the inhalation therapy of asthma in adults and in children.[1,2] BDP may hydrolyze to beclomethasone 17-monopropionate (17-BMP), beclomethasone 21-monopropionate (21-BMP), and beclomethasone (BOH). Two previous studies[3,4] have shown that the relative binding affinity of 17-BMP for the cytoplasmic glucocorticoid receptor of human lung is ~30 times greater than that of the parent drug BDP, whereas 21-BMP is practically inactive. Additionally, the affinity of BOH is 18 times less than that of 17-BMP, but still twice that of BDP.[3,4] Thus the hydrolysis of BDP is an activation step because of the formation of its active metabolite, 17-BMP. The potency of 17-BMP is ~2.5 and ~0.5 times that of the newer inhaled glucocorticoids budesonide and fluticasone propionate, respectively.[5]

The majority of an inhaled dose of chlorofluorocarbon BDP (>80%) is swallowed and may enter the systemic circulation after absorption from the gastrointestinal tract and first-pass hepatic metabolism.[6] The high therapeutic index of inhaled BDP may result from a combination of high local potency in the lung and rapid metabolic inactivation of BDP and its metabolites, especially 17-BMP, that reach the systemic circulation. Thus, characterization of the stability kinetics of beclomethasone propionate esters in human lung and plasma is of considerable importance to under-

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standing factors determining the extent of local antiinflammatory action relative to unwanted systemic activity, following inhalation of BDP.

In our previous kinetic studies of BDP in human plasma (HP),[7,8] it has been shown that 17-BMP, 21-BMP, 9β,11β-epoxy-16β-methyl-1,4-pregnadiene-17α,21-diol-3,20-dione 21-propanoate (D-3), BOH, and 9β,11β-epoxy-16β-methyl-1,4-pregnadiene-17α,21-diol-3,20-dione (D-2) are formed. The hydrolysis of BDP to 17-BMP has been demonstrated previously in human lung homogenates,[9] isolated perfused rat lungs,[10,11] and human lung 1000 × *g* supernatant (HLu).[4,12] Several major differences in the experimental methodology were previously employed in the kinetic studies of BDP in HLu, by the two separate research groups.[4,12] Magnesium chloride ($MgCl_2$) and an NADPH-generating system (co-factors), which were part of the incubation medium in the earlier study,[12] facilitate the oxidative metabolism of budesonide in human liver. However, the effect of addition of these substances on the metabolic biotransformation of beclomethasone propionate esters in HLu was not considered and is unknown.

The objectives of this study were to characterize and compare the kinetics of metabolism of BDP and 17-BMP in HLu and HP at 37 °C, and to investigate the effect of co-factors on the decomposition kinetics of BDP and 17-BMP in HLu.

EXPERIMENTAL SECTION

Materials

BDP, BOH, dexamethasone 21-acetate, sucrose, total protein reagent, protein standard solution, $MgCl_2$, monosodium d-glucose-6-phosphate (dG6P), nicotinamide adenine dinucleotide phosphate (NADP) sodium salt hydrate, glucose-6-phosphate dehydrogenase (G6PD) (Type VII from Bakers Yeast, crystalline suspension in 3.2 M ammonium sulfate solution, pH 7.0), and dichloromethane (99.9%, HPLC grade) were purchased from Sigma Chemical (St. Louis, MO). Authentic samples of BDP, 17-BMP, and BOH were gifts from Glaxo Australia (Boronia, VIC). Sodium dihydrogen orthophosphate, disodium hydrogen orthophosphate, acetic acid, and ethanol were of analytical reagent grade, whereas methanol and acetonitrile were of ChromAR HPLC grade.

Preparation of Human Lung Supernatant

Samples of human lung were obtained immediately after lung resection surgery from 12 subjects (Table 1) at three hospitals (Sydney, NSW). Written informed consent was obtained from each subject 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 pleura was removed from the parenchymal tissue, which was cut into small pieces. These small pieces were homogenized (Kinematica Polytron) in 2.5 to 5 times their volume of ice-cold isotonic 67 mM sodium phosphate buffer (pH 7.4) containing 0.21% (w/v) $NaH_2PO_4 \cdot 2H_2O$, 1.92% (w/v) $Na_2HPO_4 \cdot 12H_2O$, and 5.03% (w/v) sucrose for 3 × 10 s. All preparative steps were carried out <4 °C.

The HLu was obtained following centrifugation of the lung homogenate at 1000 × *g* (4 °C) for 10

Table 1. Demographic Data of the Subjects and Drugs Used for the 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.

min in a Sorvall RC-2B centrifuge (Amrad Pharmacia Biotech, Boronia, VIC). A separate HLu was prepared from tissue from each subject on the day of tissue collection. Total protein concentration in HLu was determined spectrophotometrically at 540 nm,[13] prior to storage at −80 °C for no longer than 1 week. HLu was diluted with ice-cold homogenizing buffer, yielding a lung protein concentration of 4 mg/mL prior to the incubation study.

Kinetic Experiments

All experiments were carried out at 37 ± 0.1 °C in borosilicate test tubes under atmospheric conditions with gentle shaking and shielded from light. Prior to commencement, HLu was equilibrated for 10 min at 37 °C. Kinetic studies were initiated by the addition of an ethanolic solution of parent drug, yielding the desired initial reactant concentration range (C_o) of 10–40 μg/mL in ethanol/media (1:99, v/v). Incubation studies in HLu were performed under two different conditions; in HLu alone under atmospheric conditions (control group), and in HLu plus co-factors (10 mM $MgCl_2$, 7.5 mM dG6P, 0.3 mM NADP, and 0.42 unit/mL G6PD) under carbogen gas (95% O_2, 5% CO_2), the so-called treatment group. Incubation studies of 17-BMP in HLu alone, in HLu containing 0.5 mM $MgCl_2$ alone, and in HLu containing various concentrations of $MgCl_2$ (0.5, 1, and 10 mM) plus the NADPH-generating system were carried out on a separate occasion. Samples (0.5 mL) were taken at appropriate time intervals and immediately frozen using dry ice/ethanol. The sampling times vary from 6 to 24 h depending on the half-lives of either BDP or 17-BMP (2–6 times half-life). Extraction and quantification of reactant and products was performed on all samples on the same day as the incubation was performed. The rate of disappearance of reactant as well as the rate of formation of products was monitored by HPLC.

Sample Preparation

Each 0.5-mL sample was spiked with 0.5 mL of a 40 μg/mL ethanolic solution of the internal standard, dexamethasone 21-acetate. Samples were extracted with 4 mL of dichloromethane for 30 min, using a roller mixer, followed by centrifugation at 2500 rpm (20 °C) for 15 min. The dichloromethane phase was collected and evaporated to dryness under nitrogen stream at 30 °C. The dried extract was reconstituted in 250 μL of 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).

Analytical Methods

The HPLC system consisted 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). The system was interfaced to a Delta 5.0 Chromatography data system from Digital Solutions (Margate, QLD).

An Alltima C_{18} (250 × 4.6 mm i.d., 5-μm particle size) HPLC column (Alltech Associates, Baulkham Hills, NSW) was used. The mobile phase, a mixture of methanol/water/acetonitrile/acetic acid (352:166:50:1, v/v) was filtered (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. The method was validated for linearity, precision, accuracy, and recovery of BDP and its degradation products.

Data Analysis

Because of the existence of nonlinearity in the kinetics of BDP and 17-BMP in homogenates of HLu, the apparent decomposition rate constants (k_{app}) were estimated from the initial slope of log–linear phase of declining concentration (minimum of eight observed concentrations) versus time plots, by least-squares fitting of rate equation using SCIENTIST® (Micromath Scientific Software, Salt Lake City, UT). All curve-fitting processes reported in this study showed good correlations ($r^2 > 0.98$) between observed values and those fitted to rate equation. The initial half-lives were calculated using the following equation: $t_{1/2} = 0.693/k_{app}$.[14] Results were expressed as the mean ± standard deviation (SD).

Statistics

Significance of a difference in means for paired and two independent samples was assessed using the paired-sample t test and Student's t test, respectively. The variability between two or more independent samples was assessed using single-factor analysis of variance (ANOVA). All values of

Table 2. Comparative Initial Half-lives of BDP and 17-BMP in HLu Alone and in HLu Containing 10 mM $MgCl_2$ and the NADPH-Generating System at 37 °C[a]

Subject number	Half-life of BDP (min)		Half-life of 17-BMP (h)	
	Control[b]	Treatment[c,d]	Control[b]	Treatment[c,d]
1	36.4 ± 0.5	44.7 ± 0.3	3.02 ± 0.02	7.4 ± 0.2
2	22.0 ± 0.3	26.0 ± 0.8	3.32 ± 0.06	8.8 ± 0.6
3	47.0 ± 0.3	51.0 ± 0.3	3.05 ± 0.02	7.9 ± 0.5
4	53.4 ± 0.3	56.4 ± 0.6	3.55 ± 0.07	9.3 ± 0.1
5	22.2 ± 0.1	59.8 ± 0.6	2.45 ± 0.03	8.0 ± 0.1
6	15.0 ± 0.3	15.6 ± 0.2	4.73 ± 0.08	11.0 ± 0.3
7	48.2 ± 0.1	51.9 ± 0.3	2.60 ± 0.04	4.7 ± 0.2
8	ND[e]	ND[e]	2.59 ± 0.01	7.7 ± 0.4
9	ND[e]	ND[e]	6.57 ± 0.08	11.4 ± 0.3
10	ND[e]	ND[e]	3.50 ± 0.04	9.4 ± 0.2
11	ND[e]	ND[e]	2.30 ± 0.05	7.0 ± 0.4
12	ND[e]	ND[e]	3.91 ± 0.06	8.8 ± 0.1

[a]Experimental data represent the mean ± SD of five determinations. [b]HLu alone. [c]HLu containing 10 mM $MgCl_2$ and the NADPH-generating system. [d]All initial half-life values observed in the treatment group were significantly different from those in the control group, by paired-sample t test. [e]ND, not determined.

p are based on two-tailed tests and p values of < 0.05 are considered significant.

RESULTS AND DISCUSSION

Degradation Reactions in HLu

The metabolic degradation pathway of BDP in HLu alone and in HLu plus co-factors involved ester hydrolysis, which is largely enzyme catalyzed.[12] Unlike the findings in HP,[7,8] 21-BMP was not detected following incubation of BDP in HLu. The rapid formation of 17-BMP following incubation of BDP in HLu alone (initial $t_{1/2} = 34.9 \pm 14.3$ min, $n = 35$, Table 2) was observed (Figure 1). The active metabolite 17-BMP was subsequently hydrolyzed with a much slower rate (initial $t_{1/2} = 3.5 \pm 1.2$ h in HLu alone, $n = 60$, Table 2) to BOH (Figures 1 and 2). The ultimate product BOH was found to be relatively stable in homogenates containing HLu alone (Figures 1 and 2) and HLu plus co-factors. The rapid decomposition of BDP in HLu may be attributed to the relatively high esterase activity for BDP in human lung.

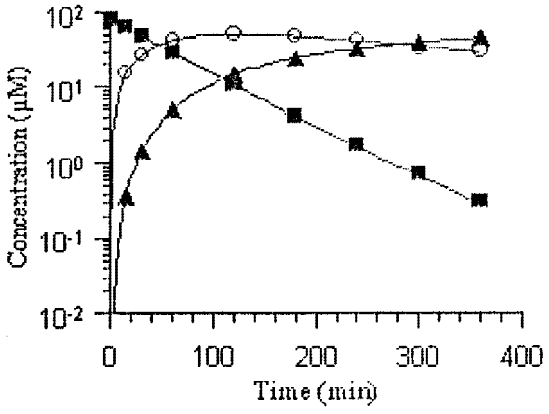


Figure 1. Typical kinetic profile of BDP (C_o = 40 μg/mL or 77 μM) and its degradation products in HLu (subject no. 3) at 37 °C. Symbols are the mean value of five determinations. Key: (■) BDP; (○) 17-BMP; (▲) BOH.

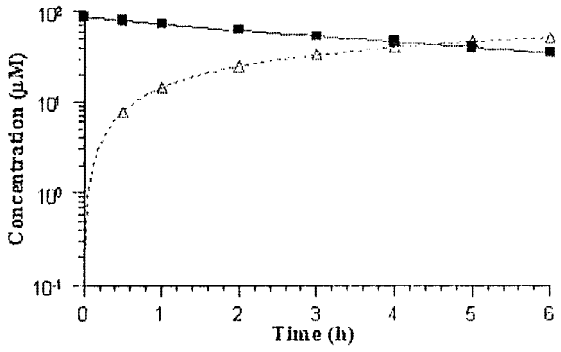


Figure 2. Typical kinetic profile of 17-BMP (C_o = 40 μg/mL or 86 μM) and its degradation product in HLu (subject no. 10) at 37 °C. Symbols are the mean value of five determinations. Key: (■) 17-BMP; (△) BOH.

Human lung contains many hydrolytic enzymes, few of which have been well characterized. The most significant esterases are epoxide hydrolase, which is localized in the microsomal fraction, and lysosomal hydrolase, which is localized in the alveolar macrophages.[15] Two previous studies[6,15] reported that inhaled glucocorticoid esters may be hydrolyzed in alveolar macrophages following absorption into the lung cells because they are rich in lysosomal esterase. The absence of metabolites resulting from the oxidative and/or reductive reactions, following incubation of BDP in both HLu alone and in HLu plus co-factors may indicate a very low activity of monooxygenase enzymes in human lung. The pulmonary distribution of cytochrome P-450 enzymes is very poor, with the exception of clara cells (nonciliated bronchiolar epithelial cells) or type II pneumocytes in the alveoli. However, because there are only few cells of this type, the overall contribution of these enzymes to the oxidative and/or reductive metabolism in the lung is relatively low.[16]

Total protein concentration in the lung supernatant reflects the esterases load and hence the hydrolyzing capacity. Thus, differences in total protein content in HLu may partially explain discrepancies in the findings reported previously.[4,12] The half-life of BDP (C_o = 50 μg/mL, HLu alone, 10 mg/mL lung protein) was calculated as 10.4 min,[4] in contrast to the findings of earlier workers[12] who reported 2.0 min (C_o = 0.05 μg/mL, HLu plus co-factors, 4 mg/mL lung protein). The difference in the initial concentration of parent drug and the presence of co-factors in the incubation medium of the earlier study may also contribute to the contradictory results of the half-life of BDP in HLu.

The half-life of BDP (2.0 min)[12] was calculated assuming BDP followed pseudo-first-order kinetics in HLu. However, we found that the kinetics of BDP and 17-BMP in HLu were nonlinear because of product inhibition and enzyme saturation. Thus, the discrepancy in the half-lives reported for BDP and 17-BMP between this study and previous findings[12] might be associated with differences in the initial concentration of parent drug.

Effect of 10 mM $MgCl_2$ and the NADPH-Generating System on the Kinetics of BDP and 17-BMP in HLu

Significantly longer initial half-lives of both BDP ($p < 0.04$) and 17-BMP ($p < 0.0005$) in HLu were observed in the treatment group compared with the control group, in all subjects (Table 2). This result is an indication that co-factors retard the decomposition rates of BDP and 17-BMP in HLu. The degree of inhibition exhibited by these species on the decomposition of BDP in HLu was much less pronounced than that of 17-BMP (Table 2). The ratios of initial half-life in the treatment group to that in the control group for BDP and 17-BMP in HLu were 1.3 ± 0.6 (n = 7) and 2.5 ± 0.5 (n = 12), respectively. Overall, the hydrolysis kinetics of BDP and 17-BMP in HLu appear to be inhibited appreciably by $MgCl_2$ with the NADPH-generating system.

It is well established that an NADPH-generating system and molecular oxygen are required for the maximum activity of liver microsomal monooxygenase enzymes.[17–19] Additionally, Mg^{+2} ion can optimize the activity of oxidative enzyme by facilitating a more rapid rate of NADPH oxidation or electron transport from NADPH to various electron acceptors.[20–22] In contrast, Mg^{+2} ion causes a decrease in the binding affinity of *N*-methylscopolamine for muscarinic receptors in rat cerebral cortex membranes.[23]

Because oxidation and/or reduction reactions were not demonstrated for BDP and 17-BMP in HLu, the inclusion of co-factors in the incubation medium is unnecessary. However, in comparative studies of drugs such as budesonide, which undergo oxidative metabolism,[12] it is understandable that the reaction mixture may contain these species. However, the results obtained underestimate the activation of BDP and inactivation of 17-BMP in the lung.

Contribution of $MgCl_2$ and/or the NADPH-Generating System to the Inhibitory Effect on the Kinetics of Beclomethasone Propionate Esters in HLu

To discriminate the relative degree of contribution of $MgCl_2$ and/or the NADPH-generating system to the inhibitory effect on the metabolic biotransformation of beclomethasone propionate esters, kinetic studies using 17-BMP as parent drug in HLu in the presence of either $MgCl_2$ alone or in combination with the NADPH-generating system were performed. The monopropionate 17-BMP, rather than BDP, was selected as parent drug for this investigation because the degree of inhibition is larger for the former. Thus, subtle alterations in the kinetic parameters will be more readily observed.

A marked increase in the initial half-life of 17-

Table 3. Contribution of $MgCl_2$ and/or the NADPH-Generating System to the Inhibitory Effect on the Kinetics of 17-BMP in HLu at 37 °C[a]

Incubation media	Initial $t_{1/2}$ (h)
HLu alone (control)	3.91 ± 0.06
HLu containing 0.5 mM $MgCl_2$ alone	4.83 ± 0.07
HLu containing 0.5 mM $MgCl_2$ plus the NADPH-generating system	7.45 ± 0.08
HLu containing 1 mM $MgCl_2$ plus the NADPH-generating system	7.6 ± 0.1
HLu containing 10 mM $MgCl_2$ plus the NADPH-generating system	7.54 ± 0.09

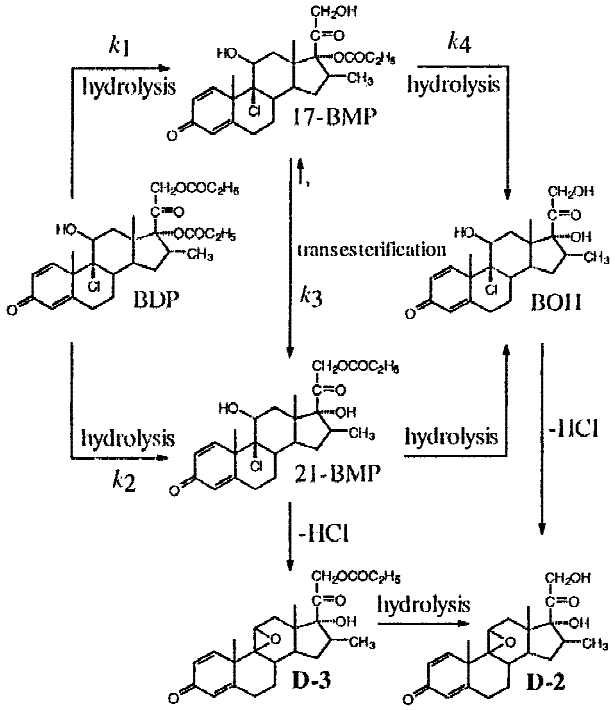
[a]Experimental data represent the mean ± SD of five determinations.

BMP was found to be primarily attributable to the NADPH-generating system, rather than $MgCl_2$ (Table 3). The ratio of initial half-life of 17-BMP estimated in HLu containing 0.5 mM $MgCl_2$ with the NADPH-generating system (treatment) to that in HLu alone (control) was 1.9 compared with the ratio of initial half-life of 17-BMP in HLu containing 0.5 mM $MgCl_2$ alone (treatment) to that in HLu alone (control) which was 1.2 (Table 3). Furthermore, there was no significant increase ($p > 0.1$) in the initial half-life of 17-BMP by further increasing the concentration of $MgCl_2$ (from 0.5 up to 10 mM) present in the reaction mixture (Table 3).

Comparative Kinetics in HP and HLu

As reported previously,[8] three reactions are involved in the metabolic pathway of BDP in HP (Figure 3); these are ester hydrolyses from diester (BDP) to monoesters (17-BMP or 21-BMP) and from monoesters (17-BMP, 21-BMP, and D-3) to the diols (BOH and D-2), the transesterification reaction of monoester 17-BMP to its isomer 21-BMP, and cyclization of the chlorohydrin moiety in 21-BMP and BOH with formation of their respective 9β,11β-epoxides D-3 and D-2 (Figure 3).[8]

In contrast to the complexity of reactions involved following incubation of BDP in HP, the metabolic pathway of BDP in HLu involved only a hydrolysis reaction. Serial formation of the intermediate metabolite 17-BMP and the ultimate product BOH was observed following incubation of BDP in HLu (Figure 1). The monoester 21-BMP was not formed following the incubation of either BDP or 17-BMP in HLu (Figures 1 and 2). In addition, reaction corresponding to the loss of hydrogen chloride was not demonstrated in HLu. It seems that the kinetics in incubation media with



k	Incubation media	
(h^{-1})	HP (n = 6)	HLu
k_1	0.037 ± 0.002	1.2 ± 0.5 (n=35)
k_2	0.027 ± 0.002	0
k_3	0.117 ± 0.001	0
k_4	0.103 ± 0.001	0.20 ± 0.07 (n=60)
k_{BDP}	0.064 ± 0.002	1.2 ± 0.5 (n=35)
$k_{17\text{-}BMP}$	0.22 ± 0.01	0.20 ± 0.07 (n=60)

Figure 3. A proposed decomposition pathway of BDP in HP and HLu.

high esterase activity such as HLu tend to favor the enzyme-catalyzed reaction pathway and eliminate reactions that are not catalyzed by enzyme, namely transesterification and loss of hydrogen chloride.

Following incubation of BDP in HP,[7,8] either 17α- or 21-BMP may be formed (Figure 3). In HP, ~58% (k_1/k_{BDP}) of BDP decomposition represents an activation process due to the formation of 17-BMP, whereas BDP was completely converted to 17-BMP in HLu (Figure 3). A reaction pathway corresponding to the inactivation of BDP to 21-BMP (k_2) was not observed in HLu (Figure 3).

Following incubation in HP,[7,8] 17-BMP may undergo two reactions; namely, hydrolysis to BOH and transesterification to 21-BMP (Figure 3). Although both pathways represent inactivation steps, BOH is still ~84 times more potent than 21-BMP with respect to the relative binding affinity for glucocorticoid receptor of human lung.[4] In HP, ~53% ($k_3/k_{17\text{-BMP}}$) of the decomposition of 17-BMP corresponds to the inactivation to 21-BMP,[8] whereas this pathway was not demonstrated in HLu (Figure 3). Because transesterification in HP favors the formation of 21-BMP (Figure 3),[8] this pathway may become an effective inactivation step for the active metabolite, 17-BMP.

There was a marked decrease in the initial half-life of BDP in HLu alone (0.6 ± 0.2 h, $n = 35$) compared with that observed in HP[7] (10.9 ± 0.4 h, $n = 6$). In contrast, no significant differences ($p > 0.3$) were demonstrated for the initial half-lives of 17-BMP estimated in HP[7] (3.0 ± 0.2 h, $n = 6$) and in HLu alone (3.5 ± 1.2 h, $n = 60$).

CONCLUSIONS

A complete and rapid activation process of BDP was demonstrated in HLu through local metabolism of BDP. In HLu, the active metabolite 17-BMP was ~6-fold more stable than its parent, BDP. This result suggests that the overall kinetics of BDP in HLu were rate-limited by the decomposition of 17-BMP. The inactive metabolite 21-BMP was not formed following incubation of BDP and 17-BMP in HLu. It may be speculated that most of the inhaled dose of BDP that reaches the lower respiratory tract is hydrolyzed to 17-BMP prior to its association with the glucocorticoid receptor within target cells.

Magnesium chloride and/or the NADPH-generating system both exhibited significant inhibition of the decomposition kinetics of BDP and 17-BMP in HLu. The respective initial half-lives for BDP and 17-BMP in HLu were prolonged by factors of 1.3 and 2.5, following the addition of co-factors into the incubation medium. It was established that the degree of inhibition by the NADPH-generating system was greater than that of $MgCl_2$.

The kinetics of metabolism of BDP and 17-BMP in HLu were qualitatively and quantitatively different from those in HP. Metabolism of BDP and 17-BMP in HLu involved only hydrolysis reactions. In contrast, three reactions were observed following incubation of BDP in HP; namely, hydrolysis, transesterification, and loss of hydrogen chloride. Effective activation of BDP to 17-BMP in HLu, together with the formation of 21-BMP in HP, might favor a high therapeutic ratio following inhalation of BDP.

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