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The role of esterases in the metabolism of ciclesonide to desisobutyryl-ciclesonide in human tissue☆

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ABSTRACT

Ciclesonide (CIC) is an inhaled glucocorticosteroid. This study aimed to identify esterases involved in the metabolism of CIC to the active metabolite desisobutyryl-ciclesonide (des-CIC), and to measure hydrolysis rates in human liver, lung and plasma and normal human bronchial epithelial (NHBE) cells *in vitro*. Ciclesonide (5 μM and 500 μM) was incubated with microsomal or cytosolic fractions from liver, lung and plasma ($n = 4$ for each) and des-CIC formation was determined by reverse-phase high-performance liquid chromatography with U.V. detection. The roles of carboxylesterase, cholinesterase and A-esterase in CIC hydrolysis were determined using a range of inhibitors. Inhibitor concentrations for liver and NHBE cells were 100 μM and 5 μM, respectively. Liver tissue had a higher activity for 500 μM CIC hydrolysis (microsomes: 25.4; cytosol: 62.9 nmol/g tissue/min) than peripheral lung (microsomes: 0.089; cytosol: 0.915 nmol/g tissue/min) or plasma (0.001 nmol/mL plasma/min), corresponding with high levels of carboxylesterase and cholinesterase in the liver compared with the lung. CIC (5 μM) was rapidly hydrolyzed by NHBE cells (~30% conversion at 4 h), with almost complete conversion by 24 h. In liver and NHBE cells, major involvement of cytosolic carboxylesterases, with some contribution by cholinesterases, was indicated. The highest level of conversion was found in the liver, the site of inactivation of des-CIC through rapid oxidation by cytochrome P450. Carboxylesterases in bronchial epithelial cells probably contribute significantly to the conversion to des-CIC in the target organ, whereas low systemic levels of des-CIC are a result of the high metabolic clearance by the liver following CIC inhalation.

1. Introduction

Inhaled corticosteroids (ICS), such as budesonide and fluticasone, are currently standard first-line therapy for the treatment of asthma [1]. They reduce bronchial hyper-responsiveness by reducing the underlying inflammation in the airways through the inhibition of inflammatory cell migration and infiltration and the release of pro-inflammatory cytokines [2–4]. The pharmacokinetic and pharmacodynamic properties of ICS determine their therapeutic efficacy as well as their propensity for local and systemic side effects [5–7].

Ciclesonide (CIC), a newer, non-halogenated topical ICS developed for the treatment of asthma, achieves high concentrations in the lung with low oral bioavailability [8–10]. Preclinical studies *in vitro* and *in vivo* show that CIC has potent anti-inflammatory activity [11]. In clinical studies, CIC significantly inhibits airway hyper-responsiveness and improves pulmonary function in patients with asthma [12–

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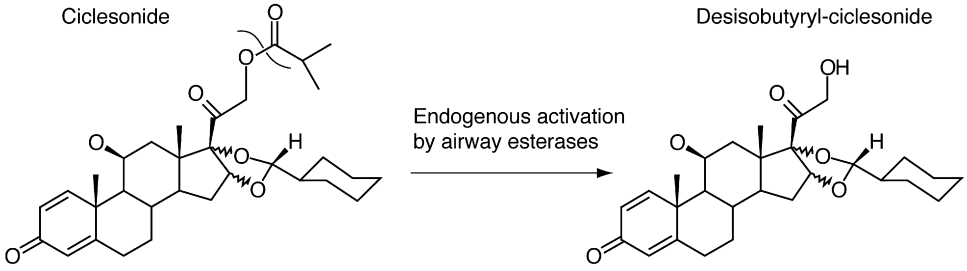

Fig. 1 – The hydrolysis of ciclesonide at position 21.

17]. Ciclesonide, the parent compound, undergoes hydrolysis by ester cleavage at the C21 position to the active metabolite, desisobutyryl-ciclesonide (des-CIC) (Fig. 1), followed by reversible formation of fatty acid esters within the lung cells [18–20]. However, the specific esterases involved in the metabolic activation of CIC, their cellular location and the location of the lipid conjugates are unknown.

The classification of esterases is based on their substrate specificity, and these enzymes are loosely classified by their interaction with organophosphates into three classes: A esterases, B esterases and C esterases [21]. B esterases (including carboxylesterases, cholinesterases and acetylcholinesterases) are serine-dependent enzymes that are important in the hydrolytic biotransformation of many therapeutic agents containing ester or amide bonds, and are inhibited by organophosphates [22–24]. They are expressed in both the endoplasmic reticulum and the cytosol of many tissues, primarily the liver [24], and are also expressed in the lung at a lower level than in the liver [25]. Human microsomal carboxylesterases (hCE) are classified as hCE-1 and hCE-2. hCE-1 is mainly expressed in the liver and at a lower level in the lung, while hCE-2 is expressed extrahepatically, although it is absent in the lung [26]. Carboxylesterases, which are expressed in human monocytes and in the cytosol from alveolar macrophages, are similar to those found in human liver [27]. Cytosolic esterases have not been fully characterized, but may be similar in many respects to the microsomal enzymes. The contribution of carboxylesterases and cholinesterases to the hydrolysis of a particular compound can be indicated by preincubation with chemical inhibitors, such as paraoxon (POX), bis(*p*-nitrophenyl)phosphate (BNPP; for carboxylesterases and cholinesterases), eserine and tetraisopropyl pyrophosphoramide (iso-OMPA; for cholinesterases only).

The aims of this study were to compare the rates of hydrolysis of CIC to des-CIC in human lung, liver, plasma and normal human bronchial epithelial (NHBE) cells in culture, and to identify the esterases involved in the hydrolysis of CIC using chemical inhibitors.

2. Materials and methods

2.1. Materials

NHBE cells with retinoic acid and bronchial epithelial growth medium (BEGM®) were purchased from BioWhittaker/Cambrex, Wokingham, UK. *p*-Hydroxymercuribenzoate (PMB), eserine, iso-OMPA, BNPP and POX were from Chem Service, Birkenhead, UK. High performance liquid chromatography (HPLC) grade acetonitrile and methanol were from Fisher Scientific, Loughborough, UK and all other chemicals, which were of analytical grade, were from Sigma–Aldrich, Gillingham, UK.

2.2. Tissue source

Human blood samples ($n = 4$) were collected by venipuncture into lithium heparin. Plasma was separated by centrifugation and stored at −70 °C. The study used four human lung and liver samples (different individuals, unidentified) from adults undergoing transplant surgery following appropriate approval by the Joint Ethical Committee of the Grampian Health Board. The liver and lung samples were free of disease as shown by histological investigation. Samples of liver and lung were flash-frozen and then stored at −70 °C prior to microsomal preparation.

NHBE cells cryo-preserved with retinoic acid (500,000 cells/1 mL ampule) were seeded into 3 × T-25 cm^2 flasks containing 5 mL BEGM. Cells were maintained for approximately 14 days in a humidified atmosphere of 5% CO_2/95% air until approximately 80% confluent. Monolayers were then harvested according to the manufacturer's protocol and the cells were counted. Aliquots of cell suspensions (500 μL) containing 150×10^3 cells/mL were seeded into 12-well plates and were maintained in BEGM in 5% CO_2/95% air for 24 h before the assay.

2.3. Preparation of liver/lung sub-cellular fractions

Approximately 1 g of human liver or lung tissue was homogenized in 10 mL of ice-cold 0.1 M KCl/0.1 M KH_2PO_4 buffer (pH 7.4) using a Polytron cell homogenizer (Kinematica AG, Switzerland) and centrifuged at 1500 × *g* for 5 min. The supernatants were centrifuged at 12,500 × *g* to remove cellular nuclei and mitochondria, and then at 100,000 × *g* for 1 h. The supernatant (cytosol) was stored at −70 °C prior to analysis. The microsomal pellets were resuspended in buffer using a glass-to-glass homogenizer and centrifuged at 100,000 × *g* for 1 h. Finally, the microsomal pellets were resuspended in 2 mL buffer and stored at −70 °C prior to analysis. The protein content of the cytosolic and microsomal fractions was determined by the bicinchoninic acid method, using bovine serum albumin as the standard [28].

2.4. Ciclesonide hydrolysis studies

2.4.1. Liver and lung tissue

All incubations were carried out using pre-determined conditions of linearity with respect to protein concentration and time at both saturating (500 μM) and low (5 μM) substrate concentrations. Incubations (duplicate) contained liver microsomal protein (0.3 mg), lung microsomal protein (0.5 mg) and liver or lung cytosolic protein (0.7 mg) in 0.5 mL of 0.1 M KCl/0.1 M KH_2PO_4 buffer (adjusted to pH 7.4 with 0.1 M KOH). The reaction was started by the addition of 5 μL CIC stock solution in dimethyl sulfoxide (DMSO) to a final CIC concentration of 5 μM or 500 μM. The incubations were conducted at 37 °C in a shaking water bath and terminated at 5 min for incubations with human liver, and 40 min for incubations with human lung. The reactions were stopped by adding an equal volume of methanol followed by rapid cooling on ice. The denatured proteins were separated by centrifugation and the supernatants were transferred to clean vials for analysis by HPLC. Control incubations from which microsomal or cytosolic protein was omitted were carried out in parallel to determine spontaneous hydrolysis of CIC.

2.4.2. Human plasma

Human plasma was incubated with CIC (5 μM and 500 μM). Incubations were carried out in duplicate and consisted of 50 μL aliquots of plasma in 0.5 mL KCl/phosphate buffer (pH 7.4). The reactions were carried out at 37 °C and terminated after 30 min by addition of an equal volume of acetonitrile. Following centrifugation, a 200-μL supernatant was analyzed by HPLC for des-CIC formation.

2.4.3. Normal human bronchial epithelial cells

Immediately before assay, the BEGM was removed from the cell monolayers, the cell surface was washed with HEPES balanced saline solution (HBSS), and 0.5 mL HBSS was added to each culture. Ciclesonide in DMSO was added to a final concentration of 5 μM to start the reaction at 37 °C. The NHBE cells and HBSS were separated and placed on ice at 1, 2, 4 and 24 h post-dose and 0.5 mL of methanol was added to stop the reaction. Following sonication and centrifugation, the supernatant was analyzed by HPLC for des-CIC formation. Control incubations, which did not contain cells, were carried out in parallel to determine the spontaneous hydrolysis of CIC.

2.5. Inhibition studies

The inhibitors and the concentrations used were selected to maximally inhibit specific esterases in each tissue as described by Brandt et al. [29].

2.5.1. Liver and lung

Inhibition profiles of CIC hydrolysis to des-CIC by human liver and lung sub-cellular fractions were determined using two concentrations of CIC (5 μM and 500 μM) and the inhibitors PMB, eserine, iso-OMPA, BNPP, POX and ethylenediaminetetraacetic acid (EDTA). Inhibitor stock (10 mM) solutions in methanol (5 μL) were added to duplicate incubations (0.5 mL) to give final inhibitor concentrations of 100 μM. Control incubations contained 5 μL methanol. The tubes were pre-incubated with inhibitor or vehicle for 10 min prior to starting the reaction with the addition of CIC. Incubations were terminated as described above and formation of des-CIC was quantified by HPLC, as before. Formation of des-CIC for each of the inhibitor treatments were compared with vehicle control measurements, and the percentage change in des-CIC formation was calculated.

2.5.2. Normal human bronchial epithelial cells

Inhibition profiles of NHBE cells were determined using CIC (5 μM) and a final concentration of 10 μM PMB, eserine, iso-OMPA, BNPP, POX and EDTA. Immediately prior to assay, the BEGM was removed from the cultures and the surface of the cell monolayers was washed with HBSS. HBSS (500 μL) and inhibitor stock solution (1 mM) in methanol (5 μL) were added to the cultures. Parallel control incubations contained 5 μL methanol. The tubes were pre-incubated with inhibitor or methanol for 10 min prior to starting the reaction with CIC. After 2 h, the cells and 0.5 mL HBSS were transferred to clean tubes and placed on ice. Methanol (500 μL) was added to the tubes, which were sonicated and centrifuged for 5 min in a microfuge. The clear supernatants were transferred to vials and formation of des-CIC was quantified by HPLC. Formation of des-CIC for each of the inhibitor treatments were compared with vehicle control measurements and the percentage change in des-CIC formation was calculated.

2.6. Kinetic studies

Kinetic characteristics for CIC hydrolysis to des-CIC were determined for human liver and lung preparations. Incubations were carried out as described above using seven CIC concentrations ranging between 5 μM and 1000 μM. The Michaelis–Menten parameters V_{max} and K_m were calculated using Eadie–Hofstee plots, and data points derived from the plots were fitted by least squares linear regression.

2.7. High performance liquid chromatography analysis

Ciclesonide and des-CIC were separated by HPLC (Kontron Instruments, Bletchley, UK) using a Luna ODS 5 μm reverse phase column (250 × 2 mm; Phenomenex, Torrance, CA) and ODS pre-column. Samples were held in a chilled autosampler in order to limit spontaneous hydrolysis of CIC before injection onto the HPLC. An 80 μL aliquot of supernatant was injected into the HPLC column and the analytes eluted by a gradient method with a flow rate of 0.2 mL/min. Acetonitrile was increased from 80% acetonitrile/20% water to 100% acetonitrile over 25 min, 100% acetonitrile for 10 min, then 80% acetonitrile/20% water until the end of the 60 min run time. Analytes were measured by detection at 242 nm with retention times of 11.3 min and 25.9 min for des-CIC and CIC, respectively.

des-CIC standards (0.5–5 μM) were prepared and stored on ice. Standards were analyzed with each batch of samples (liver, lung or NHBE cells) and des-CIC was added to incubations at the same tissue concentration as the actual incubations. Therefore, standards could be directly compared with incubated samples (liver, lung or NHBE cells), negating the need to determine recovery. Calibration curves were

prepared by linear regression analysis, using des-CIC concentration against peak area, and the calibration curve was linear over the range studied. The analytical limit of detection was 0.05 μM for des-CIC.

3. Results

3.1. Ciclesonide hydrolysis in human liver and lung sub-cellular fractions

The kinetic profile for CIC hydrolysis to des-CIC by human liver cytosol and microsomes is illustrated in Fig. 2A and B, respectively. The data were best described by bi-phasic Michaelis–Menten kinetics, with a V_{max1} value of 0.43 nmol/mg protein/min and a V_{max2} of 1.95 nmol/mg protein/min for liver cytosol; the K_{m1} and K_{m2} values were 5.4 μM and 910 μM, respectively. The V_{max1} for liver microsomes was 2.10 nmol/mg protein/min and V_{max2} was 1.09 nmol/mg protein/min, with a K_{m1} of 9.9 μM and K_{m2} of 18.7 μM.

Ciclesonide conversion to des-CIC by liver ($n = 4$) and lung ($n = 4$) microsomal and cytosolic fractions is summarized in Table 1; rates of conversion are expressed as nmol/mg protein/min and nmol/g tissue/min. Sub-cellular fractions were prepared from 1 g of liver or lung (described in Section 2) that yielded 13.3 ± 1.1 and 1.9 ± 0.35 mg microsomal protein and 134.0 ± 7.0 and 41.7 ± 7.2 mg cytosolic protein, respectively (data not shown). When activities, expressed as nmol/mg protein/min, were recalculated and expressed as nmol/g tissue/min taking these yields into consideration, the cytosol showed greater metabolic capacity.

Liver microsomal enzymes had greater activity expressed in terms of protein compared with cytosolic forms. However, the cytosol had the greatest capacity for metabolism when activity was expressed per gram of tissue. Notably, the conversion of CIC to des-CIC by microsomes and cytosol from human lung was very low. In incubations containing 500 μM CIC, only 0.05 nmol/mg protein/min was produced by lung microsomes compared with 2.02 nmol/mg protein/min by liver microsomal fractions. Similarly, liver cytosol was approximately 30 times more active than lung cytosol. It was not possible to determine the K_m or V_{max} values for CIC hydrolysis by human lung microsomes or cytosol because the activity was close to the analytical limit of detection. The formation of des-CIC in the plasma ($n = 4$) was found to be very low (9.7 ± 7.7 pmol/mL plasma/min) and was only seen following incubation with 500 μM CIC (data not shown). In incubations with 500 μM CIC, spontaneous (nonenzymatic) conversion of CIC to des-CIC was found to be $\leq$10% of the enzymatic activity; however, no spontaneous (nonenzymatic) conversion of CIC to des-CIC was observed following incubations with 5 μM CIC.

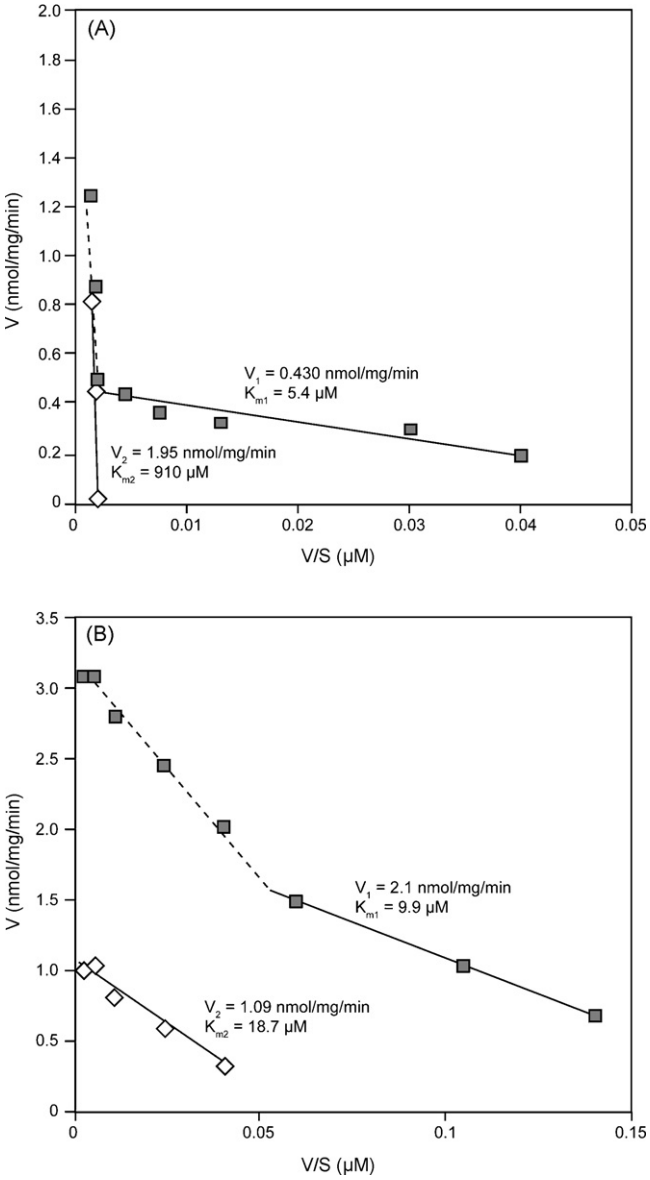

Fig. 2 – (A) Relationship between desisobutyryl-ciclesonide formation and substrate concentration in human liver cytosol. K_m and V_{max} values were determined using an Eadie–Hofstee plot. K_m = Michaelis–Menten constant; V_{max} = maximum velocity. (B) Relationship between desisobutyryl-ciclesonide formation and substrate concentration in human liver microsomes. K_m and V_{max} values were determined using an Eadie–Hofstee plot. K_m = Michaelis–Menten constant; V_{max} = maximum velocity.

3.2. Inhibition studies in liver and lung

The esterase inhibition profile in liver microsomal and cytosolic fractions is illustrated in Table 2. Inhibition of des-CIC formation by POX was nearly complete in liver microsomes, whereas inhibitions by BNPP and iso-OMPA were approximately 70% and 25%, respectively. These results indicate that the hydrolysis of CIC to des-CIC in liver microsomes is mediated primarily by carboxylesterases, with some conversion by cholinesterases. The 50% inhibition of des-CIC formation in liver microsomes by EDTA suggests the possible involvement of A-esterases, although there was little inhibition by PMB. The inhibition profile for incubations with human liver cytosol also showed nearly complete inhibition of the formation of des-CIC by POX and greater inhibition by

Table 1 – Conversion of ciclesonide to desisobutyryl-ciclesonide by human liver ($n = 4$) and lung ($n = 4$)[a]

	% Conversion to des-CIC (mean des-CIC activity ± S.E.M.)			
	Ciclesonide (5 μM)		Ciclesonide (500 μM)	
	(nmol/mg protein/min)	(nmol/g tissue/min)	(nmol/mg protein/min)	(nmol/g tissue/min)
Liver microsomes ($n = 4$)	1.02 ± 0.07	13.4 ± 0.66	2.02 ± 0.38	25.4 ± 4.7
Liver cytosol ($n = 4$)	0.25 ± 0.03	34.3 ± 4.7	0.47 ± 0.07	62.9 ± 11.4
Lung microsomes ($n = 4$)	0.086 ± 0.030	0.145 ± 0.040	0.050 ± 0.010	0.089 ± 0.18
Lung cytosol ($n = 4$)	0.008 ± 0.001	0.313 ± 0.043	0.024 ± 0.005	0.915 ± 0.051

[a] Data are expressed as mean ± S.E.M. protein for content and original weight of tissue; des-CIC = desisobutyryl-ciclesonide; S.E.M. = standard error of the mean.

Table 2 – Inhibition of ciclesonide (500 μM and 5 μM) hydrolysis by chemical inhibitors (100 μM) in human liver[a]

Inhibitor	Ciclesonide (500 μM)		Ciclesonide (5 μM)	
	Microsomes	Cytosol	Microsomes	Cytosol
Control (no inhibitor)	No inhibition	No inhibition	No inhibition	No inhibition
PMB	0	0	35	20
Eserine	63	64	96	95
Iso-OMPA	25	82	25	88
BNPP	75	80	58	95
POX	99	75	98	96
EDTA	40	50	48	10

[a] Data are expressed as percentage inhibition compared with control (mean of duplicate estimations); PMB = *p*-hydroxymercuribenzoate; iso-OMPA = tetraisopropyl pyrophosphoramide; BNPP = bis(*p*-nitrophenyl) phosphate; POX = paraoxon; EDTA = ethylenediaminetetraacetic acid.

BNPP and iso-OMPA in the cytosol than in microsomes, suggesting both carboxylesterase- and cholinesterase-mediated metabolism of CIC. Inhibition studies with human lung microsomes and cytosol were not possible because of the extremely low catalytic activity in these fractions.

3.3. *Ciclesonide hydrolysis by normal human bronchial epithelial cells*

The hydrolysis of CIC (5 μM) to des-CIC by NHBE cells was rapid and linear, with approximately 30% conversion within the first 4 h (Fig. 3). At 24 h, the concentration of des-CIC in the medium was 3.60 ± 0.33 μM, representing 96% conversion of CIC to des-CIC (Table 3).

The intracellular concentration of des-CIC in NHBE cells was calculated on the basis of the concentration of des-CIC within the cellular monolayer of the incubation, using a calculated total cell volume for each monolayer of 0.63 μL [30]. The intracellular concentration of des-CIC was higher than that measured in the culture media at all time points (Table 3). The maximum intracellular concentration of 1317 ± 158 μM was reached by 4 h. However, by 24 h, the intracellular concentration had decreased to 1006 ± 127 μM.

3.4. *Inhibition studies in normal human bronchial epithelial cells*

The inhibition of CIC in NHBE cells is shown in Table 4. Metabolism of CIC to des-CIC was inhibited 84 ± 1% by POX, 79 ± 3% by BNPP and 77 ± 3% by iso-OMPA, indicating both carboxylesterase and cholinesterase involvement. Eserine also significantly inhibited (72 ± 3%) the formation of des-CIC, but there was little effect by PMB or EDTA. This inhibition profile suggests that CIC hydrolysis was mediated by both carboxylesterases and cholinesterases, and not A esterases.

4. Discussion

This study confirms that CIC is hydrolyzed by esterases to des-CIC in human liver and lung subcellular fractions as well as in NHBE cells. In a previous study using lung and

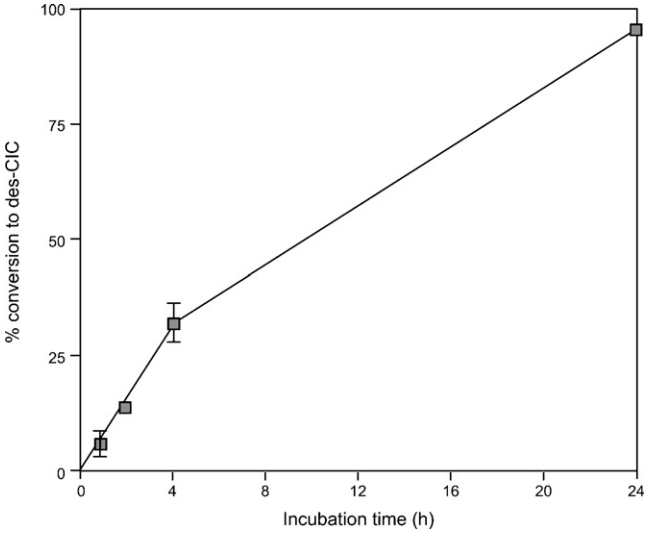

Fig. 3 – The hydrolysis of ciclesonide (5 μM) to desisobutyryl-ciclesonide by normal human bronchial epithelial cells ($n = 3$) in culture. Data are percentage conversion to des-CIC (μM), and are the mean ± standard error of the mean. des-CIC = desisobutyryl-ciclesonide.

Table 3 – Conversion of ciclesonide (5 μM) to desisobutyryl-ciclesonide by normal human bronchial epithelial cells ($n = 3$)[a]

Time (h)	Formation of des-CIC (μM) mean ± S.E.M.	
	Medium	Cells
1	0.90 ± 0.26	578 ± 201
2	1.58 ± 0.163	920 ± 233
4	2.55 ± 0.24	1317 ± 158
24	3.60 ± 0.33	1006 ± 127

[a] Data are concentrations (μM) of des-CIC (mean ± S.E.M.) in the surrounding medium and in the cells; des-CIC = desisobutyryl-ciclesonide; S.E.M. = standard error of the mean.

liver precision-cut tissue slices, CIC was metabolized in the lung to des-CIC, which subsequently underwent fatty acid conjugation, with the primary conjugate identified as the oleate of des-CIC [19]. In liver slices, CIC was metabolized to des-CIC with further metabolism to several inactive polar metabolites following oxidation. Cytochrome P450 3A4 was identified as the Phase I enzyme involved in the metabolism of des-CIC to its polar metabolites [31]; however, the specific esterases involved in the hydrolysis of CIC to des-CIC and their compartmental location were not determined. No inactive polar metabolites were detected in the lung slices.

The human liver has a high capacity to hydrolyze CIC, which likely corresponds with the high levels of carboxylesterase and cholinesterase in this organ. The inhibition profile for hydrolysis of CIC by liver subcellular fractions indicates that the reaction is mediated by both cytosolic and microsomal carboxylesterases, with some involvement of cholinesterases, and that the high-affinity cytosolic carboxylesterase isoform(s) involved may be similar to cholesterol hydrolase A found in rats [32]. It is not surprising that the hydrolysis of CIC to des-CIC was mediated by both carboxylesterases and cholinesterases because the catalytic triad of amino acid residues (serine, glutamic acid, histidine) is highly conserved in the active site among the serine hydrolase superfamily of enzymes [24]. With the recent elucidation of the crystal structure of hCE-1, a major carboxylesterase in human liver, a greater understanding of its active site may help identify substrate specificities of the carboxylesterase enzymes and, therefore, the specific isoenzymes responsible for the metabolism of CIC [33].

Human peripheral lung tissue had a lower hydrolytic capacity compared with human liver tissue, which was not unexpected because it is known that there is a lower expression of esterases in the lung compared with the liver [25]. CIC has been previously shown to be rapidly hydrolyzed to des-CIC in human lung slices [34], where all the pulmonary cell types were present, as well as in human nasal epithelial (NHBE) cells [35]. We suggest that the very small lung samples used in the current study (peripheral/tip) were less representative than lung slices as they did not contain sufficient cells typically present in the upper airways (i.e. nasal and bronchial cells).

Incubation of NHBE cells with 5 μM CIC showed a high rate of hydrolysis at 4 h post-dose. By 24-h post-dose the conversion of CIC to des-CIC was almost complete. The esterase inhibition profile was consistent with the involvement of carboxylesterases and cholinesterases at these concentrations and was similar to the high-affinity component in human liver cytosol. It is currently not known whether the location of the esterases mediating CIC hydrolysis in NHBE cells is cytosolic or microsomal.

In conclusion, these studies show rapid conversion of CIC to des-CIC by human liver and NHBE cells in culture. The highest levels of conversion of CIC to des-CIC were seen in the liver, which is also the relevant site for inactivation by metabolism of des-CIC through rapid oxidation by cytochrome P450. The high metabolism seen in the liver results in low systemic levels of active des-CIC following administration of CIC [10]. The low levels of carboxylesterase in human plasma [25], together with the high protein binding of 99% [36], suggest that there will be negligible systemic conversion of CIC to des-CIC following administration of CIC, as confirmed *in vitro* in this investigation using human plasma. The high levels of conversion of CIC to des-CIC by carboxylesterases present in the bronchial epithelial cells may contribute significantly to the local activation by hydrolysis of an inhaled dose of CIC to des-CIC in the target organ.

Table 4 – Inhibition of ciclesonide (5 μM) hydrolysis by chemical inhibitors (10 μM) in normal human bronchial epithelial cells ($n = 3$)[a]

Inhibitor	Assay 1	Assay 2	Assay 3	Mean ± S.E.M.
Control (no inhibitor)	No inhibition	No inhibition	No inhibition	No inhibition
PMB	20	34	10	21 ± 7
Eserine	75	75	65	72 ± 3
Iso-OMPA	70	80	80	77 ± 3
BNPP	74	79	85	79 ± 3
POX	85	85	82	84 ± 1
EDTA	14	25	20	20 ± 3

[a] Data are expressed as percentage inhibition compared with control (mean of duplicate estimations); S.E.M. = standard error of the mean; PMB = *p*-hydroxymercuribenzoate; iso-OMPA = tetraisopropyl pyrophosphoramide; BNPP = bis(*p*-nitrophenyl) phosphate; POX = paraoxon; EDTA = ethylenediaminetetraacetic acid.

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