BIOPHARMACEUTICS & DRUG DISPOSITION
Biopharm. Drug Dispos. **28**: 59–64 (2007)
Published online in Wiley InterScience
(www.interscience.wiley.com) **DOI**: 10.1002/bdd.531

In Vitro Activation of the Corticosteroid Ciclesonide in Animal Nasal Mucosal Homogenates

H. Sato[a], R. Nave[b,*], T. Nonaka[a], T. Mochizuki[a], S. Takahama[a] and S. Kondo[a]
[a] *Teijin Institute for Biomedical Research, TEIJIN Pharma Limited, Tokyo, Japan*
[b] *ALTANA Pharma AG, Konstanz, Germany*

ABSTRACT: Ciclesonide, a new corticosteroid for allergic rhinitis, is administered as an inactive parent compound that is converted by esterases to the pharmacologically active metabolite, desisobutyryl-ciclesonide (des-CIC). This study investigated the *in vitro* activation of ciclesonide in nasal mucosa of multiple animal species. Nasal mucosal homogenates from rats, guinea-pigs, rabbits and dogs were incubated with ciclesonide 0.5 μmol/l (0.271 μg/ml) or 5 μmol/l (2.71 μg/ml) for up to 120 min. Concentrations of ciclesonide and des-CIC were measured by high-performance liquid chromatography with tandem mass spectrometry. Ciclesonide was metabolized to des-CIC in nasal mucosal homogenates of each species. The initial velocities of des-CIC formation ranged from 0.0038 to 0.0150 nmol/min/mg protein and 0.0319 to 0.0983 nmol/min/mg protein in nasal mucosal homogenates incubated with ciclesonide 0.5 μmol/l and 5 μmol/l, respectively. Furthermore, the initial velocities of ciclesonide metabolism ranged from 0.0032 to 0.0142 nmol/min/mg protein and 0.0445 to 0.1316 nmol/min/mg protein in nasal mucosal homogenates incubated with ciclesonide 0.5 μmol/l and 5 μmol/l, respectively. This study confirms that ciclesonide is converted to des-CIC in nasal mucosal homogenates without any marked differences among animal species. Copyright © 2007 John Wiley & Sons, Ltd.

Key words: allergic rhinitis; esterases; intranasal corticosteroids; nasal mucosa

Introduction

Allergic rhinitis (AR) affects an estimated 20–40 million Americans [1]. This disease is characterized by inflammation of the nasal mucosa that follows a 2stage process. During the early phase of the allergic response, mast cells release a variety of factors (e.g. histamine, cysteinyl leukotrienes, cytokines) that elicit symptoms of sneezing, itching and rhinorrhea [2]. Factors released by mast cells during the early phase trigger the activation and infiltration of inflammatory cells into the nasal mucosa during the late-phase response, resulting in nasal congestion and obstruction.

Among the treatment options for AR, intranasal corticosteroids (INCS) are the most effective and relieve nasal congestion, a symptom that is not adequately controlled by oral antihistamines [3,4]. Intranasal corticosteroids deliver medication to the site of local inflammation, the nasal mucosa. Specifically, INCS inhibit the expression and release of proinflammatory cytokines, thereby reducing the inflammatory response of mast cells, eosinophils, interleukin-4 (IL-4) immunoreactive cells and Th2 cells [2,3,5].

Ciclesonide is a corticosteroid that was initially developed for the treatment of asthma. An intranasal formulation of ciclesonide has recently been developed for the treatment of AR.

*Correspondence to: ALTANA Pharma AG, Byk-Gulden-Str. 2, 78467 Konstanz, Germany. E-mail: ruediger.nave@altanapharma.com

Received 31 March 2006
Revised 28 September 2006
Accepted 4 October 2006

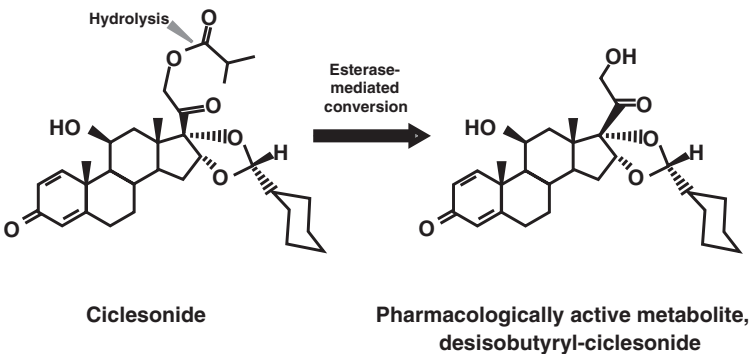

Figure 1. Ciclesonide is converted to the active metabolite, desisobutyryl-ciclesonide, by endogenous esterases of the upper and lower airways. Adapted with permission of S. Karger AG [6]

Ciclesonide is administered as an inactive parent compound that is converted by endogenous esterases of the upper and lower airways to the pharmacologically active metabolite, desisobutyryl-ciclesonide (des-CIC; Figure 1) [6]. The relative glucocorticoid receptor binding affinity of des-CIC is 100-fold greater than the parent compound (relative glucocorticoid receptor binding affinities are 1200 and 12, respectively; dexamethasone reference is 100) [7]. Activation of ciclesonide was demonstrated in rat lungs *in vivo* [8] and in human lung tissue and cells *in vitro* [9,10]. Further specific features of ciclesonide are its low oral bioavailability (<1%) [11] and high serum protein binding (99%) in circulation [12]. Ciclesonide for intranasal use is formulated in a hypotonic suspension, which has been shown in preclinical *in vivo* models to provide enhanced tissue uptake when compared with a traditional isotonic formulation [13].

The objective of this study was to confirm the *in vitro* biotransformation of ciclesonide to the active metabolite, des-CIC, in nasal mucosal tissue homogenates from rats, guinea-pigs, rabbits and dogs. Furthermore, the pharmacokinetic parameters of ciclesonide metabolism were compared among animal species tested.

Methods

Animals

Male Sprague-Dawley rats (9 weeks old; $n = 26$) and male Hartley guinea-pigs (11 weeks old; $n = 15$) were obtained from Charles River Japan Inc. (Yokohama, Japan). Male Japanese White rabbits (18 weeks old; $n = 3$) were supplied by Japan SLC Inc. (Shizuoka, Japan). Two 16-month-old male beagles were provided by Naruku Corp. (Chiba, Japan). Food and water for all animals were provided *ad libitum* during the acclimation period. Specific pathogen-free animals (rats, guinea-pigs and rabbits) were housed in clean animal rooms, and dogs were housed in conventional animal rooms at the Teijin Institute for Biomedical Research in accordance with national guidelines and legal regulations.

Collection of nasal mucosa

Rats and guinea-pigs were anesthetized with diethyl ether, rabbits with pentobarbital sodium (Nembutal® injection, Dainabot, Osaka, Japan), and dogs with pentobarbital sodium subsequent to xylazine hydrochloride (ROMPUN® 2% injection, Bayer, Leverkusen, Germany) pretreatment. Animals were exsanguinated and decapitated. The skull was divided by a sagittal incision, and the nasal mucosal tissue (ventral nasal concha, middle nasal meatus, choana, and olfactory mucosa) was immediately collected.

Excised tissue was weighed, minced and suspended in a 10-fold volume of ice-cold incubation buffer (0.1 M Tris-HCl, pH 7.4 containing 0.2 M sucrose). Tissue was homogenized using a Teflon digital homogenizer (Asone, Osaka, Japan), then centrifuged ($1000 \times g$) for 10 min at 4 °C. The supernatant was stored at –80 °C until further analysis. *In vitro*

DOI: 10.1002/bdd

assays were performed within 8 days of tissue collection.

Protein concentration in nasal mucosal homogenates was quantified using the bicinchoninic acid (BCA) colorimetric assay (BCA Protein Assay Kit, Pierce Biotechnology, Inc, Rockford, Ill, USA) [14] with bovine serum albumin as a reference standard. Homogenates were diluted to 1 mg protein/ml as necessary.

Experimental procedures

Nasal mucosal homogenates from each animal were pooled by species. Nasal mucosal homogenates (490 µl) were preincubated at 37 °C for 15 min. The reaction was initiated by addition of 10 µl of ciclesonide (0.5 or 5 µmol/l) or des-CIC (0.5 or 5 µmol/l) solutions dissolved in ethanol. Nasal mucosal homogenates were incubated for 0, 15, 30, 60 and 120 min with ciclesonide or 0 and 120 min for des-CIC. Reactions were terminated by the addition of 1.95 ml of ice-cold ethanol. Subsequently, 50 µl of deuterium-labeled des-CIC (internal standard) was added to the reaction. Samples were centrifuged ($2620 \times g$) for 10 min at 4 °C. Supernatants were stored at −20 °C until analysis.

Concentrations of ciclesonide and des-CIC were measured using reversed phase HPLC (Agilent 1100, Agilent, Tokyo, Japan) with MS/MS detection (PE Sciex API 3000, Applied Biosystems, Tokyo, Japan). Lower limits of quantification were 1.0 ng/ml for ciclesonide and 0.5 ng/ml for des-CIC. The molecular weight of ciclesonide and des-CIC are 540.7 and 470.6, respectively.

Statistical analysis

Peak area ratios of ciclesonide and des-CIC relative to the internal standard were calculated, and a calibration curve was generated for each analyte. Values of ciclesonide and des-CIC determined from the calibration curve were divided by protein concentration to yield a concentration in nmol/mg protein. Calculations were performed using Analyst version 1.1 (PE Sciex) and Microsoft Excel 2000. The initial reaction velocities of ciclesonide and des-CIC formation were calculated by dividing the decrease in ciclesonide or increase in des-CIC during the first 15 min of incubation by the incubation period. The *in vitro* half-lives of ciclesonide and des-CIC were determined from the log-linear region of the concentration versus time curve.

Results

Ciclesonide was converted to des-CIC in the nasal mucosa homogenates from all four animal species tested at both initial concentrations of ciclesonide (Table 1; Figure 2). The metabolism of ciclesonide followed first-order reaction kinetics. Concentrations of des-CIC in homogenates

Table 1. Kinetic parameters of ciclesonide and desisobutyryl-ciclesonide

	Ciclesonide		Desisobutyryl-ciclesonide	
	5 µmol/l[a]	0.5 µmol/l[a]	5 µmol/l[a]	0.5 µmol/l[a]
Initial velocity (nmol/min/mg protein)				
Rabbit	0.1231	0.0142	0.0983	0.0150
Rat	0.1316	0.0107	0.0641	0.0053
Guinea-pig	0.0445	0.0084	0.0455	0.0058
Dog	0.0476	0.0032	0.0319	0.0038
Half-life (min)				
Rabbit	71.2	34.0	—	—
Rat	58.8	57.8	—	—
Guinea-pig	100.9	104.1	—	—
Dog	106.2	95.9	—	—

[a] Initial substrate (ciclesonide) concentration.

DOI: 10.1002/bdd

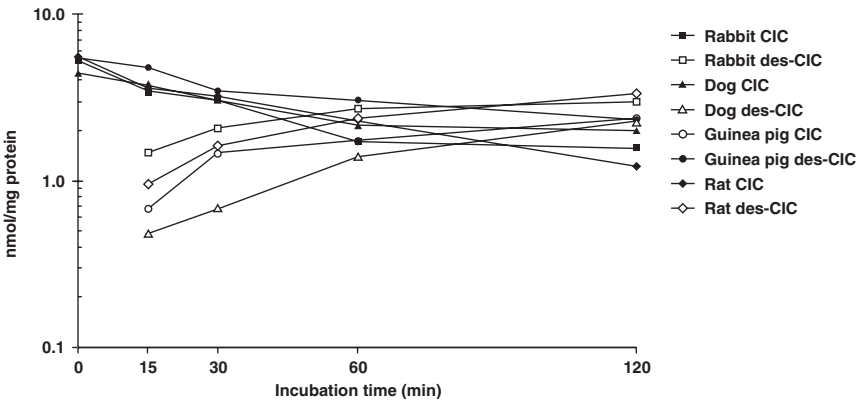

Figure 2. Concentrations of ciclesonide and desisobutyryl-ciclesonide over time in rabbit, dog, guinea-pig, and rat nasal homogenates during incubation with ciclesonide at an initial concentration of 5 μmol/l. CIC, ciclesonide; des-CIC, desisobutyryl-ciclesonide

increased with duration of ciclesonide incubation. Incubation of des-CIC with nasal mucosal homogenates for 120 min at 37 °C yielded concen trations of des-CIC similar to the initial concentrations of 5 or 0.5 μmol/l (data not shown), demonstrating the metabolic stability under the tested conditions. However, there was no formation of des-CIC fatty acid conjugates, possibly because of missing cofactors in the test system.

Initial velocities of des-CIC formation during the first 15 min of exposure of nasal mucosal homogenates to ciclesonide 5 μmol/l were 0.0983, 0.0641, 0.0455 and 0.0319 nmol/min/mg protein for rabbit, rat, guinea-pig and dog, respectively (Table 1). Initial des-CIC formation velocities during incubation with ciclesonide 0.5 μmol/l were approximately 10-fold lower, ranging from 0.0038 to 0.0150 nmol/min/mg protein. The half-lives of ciclesonide for all species and initial concentrations occurred within the 120 min incubation period. After homogenate incubation with ciclesonide 5 μmol/l, the half-lives of ciclesonide were 71.2, 58.8, 100.9 and 106.2 min in rabbit, rat, guinea-pig and dog nasal mucosa homogenates, respectively (Table 1). The half-lives of ciclesonide after incubation with the lower concentration of ciclesonide (0.5 μmol/l) were generally similar, except in rabbit nasal mucosa, in which the half-life decreased by approximately 50% compared with the higher ciclesonide concentration (34.0 min; Table 1).

Metabolism of ciclesonide and formation of des-CIC were slightly higher in rat and rabbit compared with dog and guinea-pig (Table 1). However, differences among species were not pronounced.

Discussion

In this study, ciclesonide was converted to des-CIC in nasal mucosa homogenates from all four animal species tested, and the ciclesonide half-life was <120 min. This study suggests that the activation of ciclesonide by ester hydrolysis is present in the nasal mucosa among animal species. The results of this study are also consistent with the previous report of ciclesonide activation in rabbit nasal mucosal tissue [13].

The concentration of the pharmacologically active metabolite, des-CIC, did not change appreciably during the 120 min incubation with nasal mucosa homogenates. The total amounts of des-CIC and ciclesonide were slightly lower after 120 min of incubation compared with the initial amount of ciclesonide ($\geqslant 86\%$), possibly because of the conversion of des-CIC to des-CIC fatty acid conjugates. Although this study did not investigate fatty acid conjugation, previous studies demonstrated that esterification of des-CIC at the C-21 position resulted in the formation of oleate and palmitate esters [8]. Fatty acid conjugation of inhaled corticosteroids is thought

DOI: 10.1002/bdd

to increase drug retention in the lung [15,16] and may, therefore, also increase the retention of intranasal corticosteroids in the nasal mucosa. Conversion of des-CIC to des-CIC fatty acid conjugates is reversible, indicating that stored fatty acid conjugates can revert to the pharmacologically active moiety, des-CIC [17].

The rate of ciclesonide metabolism and des-CIC formation varied only slightly among species. Based on data in the liver and normal human bronchial epithelial cells, the metabolism of ciclesonide to des-CIC is catalysed primarily by carboxylesterases and to a lesser degree by cholinesterases [9]. Mammalian species, including humans, have multiple isoforms of carboxylesterase that are highly homologous, with conserved amino acid sequences at the active site [18]. There are, however, notable species differences in tissue distribution of carboxylesterases in the olfactory region [19]. Similarly, plasma cholinesterase (butyrylcholinesterase) is highly conserved in mammalian species ($\geqslant$91.5%), although its levels and activity are highly variable among vertebrate species [20]. Therefore, differences in ciclesonide metabolism among the four species tested in this study may reflect differences in isozyme activity and tissue distribution of carboxylesterase and cholinesterase.

Metabolism of ciclesonide in the nasal epithelia was predicted based on the conversion of ciclesonide to des-CIC by endogenous esterases of the lung [9]. In order for ciclesonide to be an effective treatment for AR, it was important to confirm that the pharmacologically active metabolite would be formed in the target tissue, the nasal mucosa. Therefore, this study forms the basis for further assessments of ciclesonide metabolism *in vivo* and in human nasal tissue.

In conclusion, ciclesonide was metabolized to des-CIC *in vitro* in the nasal mucosa of various species, with only slight variations in metabolism rates. These data along with the conservation of the metabolic enzymes involved in ciclesonide hydrolysis to des-CIC among mammalian species indicate that ciclesonide is likely to be converted to the pharmacologically active metabolite, des-CIC, in human nasal mucosa. The recent report that intranasal administration of ciclesonide 200 μg once daily provided effective symptom relief in patients with AR [21] suggests that ciclesonide is converted to des-CIC in human nasal mucosa. The presence of active metabolite in the nasal mucosa supports the continued clinical development of ciclesonide for the treatment of AR.

Acknowledgements

This study was sponsored by TEIJIN Pharma Limited, Tokyo, Japan.

References

1. Skoner DP. Allergic rhinitis: definition, epidemiology, pathophysiology, detection, and diagnosis. *J Allergy Clin Immunol* 2001; **108**(Suppl): S2–S8.
2. Quraishi SA, Davies MJ, Craig TJ. Inflammatory responses in allergic rhinitis: traditional approaches and novel treatment strategies. *J Am Osteopath Assoc* 2004; **104**(Suppl 5): S7–S15.
3. Rachelefsky GS. Pharmacologic management of allergic rhinitis. *J Allergy Clin Immunol* 1998; **101**: S367–S369.
4. Dykewicz MS, Fineman S. Executive summary of joint task force practice parameters on diagnosis and management of rhinitis. *Ann Allergy Asthma Immunol* 1998; **81**: 463–468.
5. Mygind N, Nielsen LP, Hoffmann HJ, *et al*. Mode of action of intranasal corticosteroids. *J Allergy Clin Immunol* 2001; **108**(Suppl): S16–S25.
6. Dietzel K, Engelstätter R, Keller A. Ciclesonide: an on-site-activated steroid. In *New Drugs for Asthma, Allergy and COPD*, Progress in Respiratory Research, vol. 31, Hansel TT, Barnes PJ (eds). Karger: Basel, 2001; 91–93.
7. Stoeck M, Riedel R, Hochhaus G, *et al*. *In vitro* and *in vivo* anti-inflammatory activity of the new glucocorticoid ciclesonide. *J Pharmacol Exp Ther* 2004; **309**: 249–258.
8. Nave R, Meyer W, Fuhst R, Zech K. Formation of fatty acid conjugates of ciclesonide active metabolite in the rat lung after 4-week inhalation of ciclesonide. *Pulm Pharmacol Ther* 2005; **18**: 390–396.
9. Mutch E, Nave R, Zech K, Williams FM. Esterases involved in the hydrolysis of ciclesonide in human tissues [abstract]. *Eur Respir J* 2003; **22**(Suppl 45): 267s–268s. Abstract P1749.
10. Nave R, Fisher R, Zech K. *In vitro* metabolism of ciclesonide in human lung and liver precision-cut tissue slices. *Biopharm Drug Dispos* 2006; **27**: 197–207.
11. Nave R, Bethke TD, van Marle SP, Zech K. Pharmacokinetics of [14C]ciclesonide after oral and intravenous administration to healthy subjects. *Clin Pharmacokinet* 2004; **43**: 479–486.
12. Rohatagi S, Luo Y, Shen L, *et al*. Protein binding and its potential for eliciting minimal systemic side effects with a

DOI: 10.1002/bdd

novel inhaled corticosteroid, ciclesonide. *Am J Ther* 2005; **12**: 201–209.
13. Wingertzahn MA, Sato H, Nave R, Nonaka T, Mochizuki S, Takahama S. Comparison of nasal tissue concentrations in rabbits following administration of hypotonic and isotonic ciclesonide suspensions [abstract]. *J Allergy Clin Immunol* 2005; **115**(Suppl): S126. Abstract 503.
14. Smith PK, Krohn RI, Hermanson GT, *et al*. Measurement of protein using bicinchoninic acid. *Anal Biochem* 1985; **150**: 76–85.
15. Tunek A, Sjodin K, Hallstrom G. Reversible formation of fatty acid esters of budesonide, an antiasthma glucocorticoid, in human lung and liver microsomes. *Drug Metab Dispos* 1997; **25**: 1311–1317.
16. Miller-Larsson A, Mattsson H, Hjertberg E, Dahlbäck M, Tunek A, Brattsand R. Reversible fatty acid conjugation of budesonide. Novel mechanism for prolonged retention of topically applied steroid in airway tissue. *Drug Metab Dispos* 1998; **26**: 623–630.
17. Wingertzahn M, Nonaka T, Mochizuki R, Sato H, Kondo S. Reversibility of desisobutyryl-ciclesonide conversion to fatty acid conjugates [abstract]. *J Allergy Clin Immunol* 2006; **117**: S167. Abstract 652.
18. Satoh T, Hosokawa M. Molecular aspects of carboxylesterase isoforms in comparison with other esterases. *Toxicol Lett* 1995; **82–83**: 439–445.
19. Mainwaring G, Foster JR, Lund V, Green T. Methyl methacrylate toxicity in rat nasal epithelium: studies of the mechanism of action and comparisons between species. *Toxicology* 2001; **158**: 109–118.
20. Arpagaus M, Chatonnet A, Masson P, *et al*. Use of the polymerase chain reaction for homology probing of butyrylcholinesterase from several vertebrates. *J Biol Chem* 1991; **266**: 6966–6974.
21. Ratner PH, van Bavel JH, Hampel F, *et al*. Dose-dependent effectiveness of ciclesonide nasal spray in the treatment of seasonal allergic rhinitis (SAR) [abstract]. *J Allergy Clin Immunol* 2004; **113**: S28. Abstract 14.

DOI: 10.1002/bdd