

A clinical pharmacokinetic study comparing two azelastine hydrochloride nasal formulations in a single-dose design

Daniel Du · Darren Targett · Erhard Stolberg ·
Alessandra Canali

Received: 18 July 2012 / Accepted: 3 May 2013 / Published online: 17 May 2013
© Springer-Verlag France 2013

Abstract Azelastine hydrochloride is a potent second-generation antihistamine, available in Europe and the USA as a nasal spray formulation for the treatment of allergic rhinitis symptoms. GlaxoSmithKline (GSK) Consumer Healthcare has developed a new nasal formulation of azelastine hydrochloride. The present study was aimed at comparing the clinical pharmacokinetic profiles and assessing the bioequivalence of the new formulation of azelastine hydrochloride with a marketed reference nasal spray product. This was a randomized, two-way crossover, two-stage, single-dose pharmacokinetic study with 2 weeks washout between the two treatment periods. A dosage of 0.28 mg of the test and reference products was administered as a single dose to healthy volunteers according to the crossover design. Twenty-three subjects (15 subjects from stage 1 and 8 subjects from stage 2) were enrolled in the study. Adjusted mean values for AUC_{0-t} were 1,526.8 h pg/mL for the test drug and 1,441.5 h pg/mL for the reference drug; for C_{max} the values were 61.59 pg/mL for the test drug and 58.21 pg/mL for the reference drug. The 94.12 % CI of geometric mean ratios (test/reference) were 0.99–1.13 and 0.95–1.18 for AUC_{0-t} and C_{max} . This

met the predefined criteria for bioequivalence between test and reference drugs. Secondary pharmacokinetic parameters for azelastine and for the metabolite desmethyl azelastine, $AUC_{(0-\infty)}$ and t_{max} , were numerically similar between the two study treatments. Both test and reference azelastine hydrochloride formulations were well tolerated at single dose. This study demonstrated the bioequivalence between the new azelastine hydrochloride nasal spray formulation and the marketed reference Allergodil® after single-dose administration.

Keywords Azelastine · Antihistamine · Bioequivalence · Nasal spray · Allergic rhinitis · Pharmacokinetics

1 Introduction

Allergic rhinitis is reported to affect 20–40 % of the population worldwide, with an increasing prevalence over recent decades in both children and adults (Wallace et al. 2008). It has been identified as one of the top ten reasons for visits to primary care clinics. Although symptoms are not life threatening, allergic rhinitis can significantly affect the quality of life and work productivity of patients, with substantial health care and social economic impacts (Bousquet et al. 2008). Histamine is the major inflammatory mediator in the pathophysiology of allergic rhinitis symptoms and acts in the nose primarily via type 1 histamine (H_1) receptors. H_1 -antihistamines act by blocking the H_1 -histamine receptors, thus having anti-inflammatory effects. Local topical nasal antihistamine spray has the advantage of delivering drug directly into the nose, so that high local concentrations of drug can be achieved more effectively at the target organ with minimized systemic side effects.

D. Du (✉)
Medical Affairs, GSK Consumer Healthcare, 1500 Littleton Rd,
Parsippany, NJ 07054, USA
e-mail: daniel.y.du@gsk.com

D. Targett · A. Canali
GSK Consumer Healthcare, Via Zambelletti snc,
20021 Baranzate, MI, Italy
e-mail: alessandra.canali@gsk.com

E. Stolberg
Nuvisan GmbH, Wegenerstrasse 13, 89231 Neu-Ulm, Germany
e-mail: erhard.stolberg@t-online.de

Azelastine hydrochloride, a phthalazinone derivative, is a potent second-generation antihistamine that has shown H₁-receptor antagonist activity in isolated tissues, animal models and humans with ten times greater potency than chlorphenamine (Casale 1989; Zechel et al. 1981). Azelastine also exerts its anti-allergic effect through inhibition of synthesis or release of other inflammatory mediators such as leukotrienes, kinins, superoxide/free oxygen radicals, cytokines and ICAM-1 expression and the prevention of inflammatory cell activation and/or migration (Chand et al. 1989; Ito et al. 1998; Ciprandi et al. 1996; Shinoda et al. 1997; Nieber et al. 1993). Azelastine hydrochloride is available in Europe and the USA as a nasal spray formulation. In the USA, azelastine nasal spray is indicated for the treatment of seasonal allergic rhinitis (SAR) and non-allergic vasomotor rhinitis, while in Europe it is indicated for the treatment of SAR and perennial allergic rhinitis (PAR).

A new nasal formulation of azelastine hydrochloride has been developed for the indication of allergic rhinitis. The present study aimed at comparing the clinical pharmacokinetic (PK) profiles and assessing the bioequivalence of the new formulation of azelastine hydrochloride (Glaxo-SmithKline Consumer Healthcare S.p.A., Italy) with a marketed reference nasal spray product (Allergodil® MEDA Pharma S.p.A., Italy).

2 Materials and methods

2.1 Study protocol

The approval of the study protocol by the regional ethics committee and by the competent national authority (Germany Federal Institute for Drugs and Medical Devices [BfArM]) was obtained before the start of the study. The study was conducted from 2 August 2010 to 27 December 2010 at Nuvisan GmbH Clinical Pharmacology Centre, Neu-Ulm, Germany, in accordance with the 2008 Declaration of Helsinki and ICH Guideline for Good Clinical Practice. Written informed consent was obtained from each study participant before screening procedures.

2.2 Subjects

Volunteers were recruited from the study centre's subject database and through local advertisement. Healthy male and female non-smoking (for at least 3 months prior to the screening visit) volunteers, aged 18–55 years (inclusive), with a body mass index (BMI) between 18.5 and 30 kg/m², were eligible for inclusion. Females with childbearing potential had to use effective contraceptive methods (e.g. implants, injectables, combined oral contraceptives,

selected IUDs, sexual abstinence or vasectomized partner) for at least 1 month prior to the start of the study. Volunteers were excluded from the study for the following reasons: unable to provide consent; had abnormal findings on electrocardiography or vital signs; had positive serum test results for hepatitis B, hepatitis C or HIV at screening; had a history of addiction or significant use of drugs, alcohol or caffeine; had a history of any allergic reactions or ascertained or presumptive hypersensitivity to the active pharmaceutical ingredient or formulation excipients. Volunteers were also excluded if they had a history of any clinically significant renal, hepatic, gastrointestinal, cardiovascular, respiratory, skin, haematological, endocrine or neurological disease that could interfere with the aim of the study, and/or had received medication within 14 days of the screening visit. Additional exclusion criteria were current nasal abnormalities that could impact on the application or action of the investigational products, e.g. current perennial or seasonal allergic rhinitis, nasopharyngitis, cold or flu; any clinically significant nasal anatomic abnormalities, such as severely deviated nasal septum, nasal polyps, completely blocked nostril or history of nasal major surgery.

2.3 Study design and sample size determination

This was a randomized, two-way crossover, two-stage, single-dose pharmacokinetic study with 2 weeks washout between the two treatment periods. The single-dose regimen was chosen to assess the bioequivalence of the test versus the reference product, in compliance with the current Committee for Proprietary Medicinal Products (CPMP) guidelines for PK and bioequivalence studies (Guideline on the investigation of bioequivalence. CPMP/EWP/QWP/1401/98, Rev 1/Corr 20th January 2010). An adaptive two-stage approach was applied in this study for subject sample size adjustment because there was little data available to make an a priori sample size calculation. In stage 1, a total of 15 subjects were randomized to get a minimum of 12 evaluable subjects. Provided the intra-subject coefficient of variation (CV) was no higher than 15 %, it was determined that 12 evaluable subjects would have greater than 80 % power to demonstrate bioequivalence in stage 1, assuming the true ratio of geometric means was 0.95. The overall sample size requirements for stage 2 was calculated based on the intra-subject CV observed in stage 1.

The following adaptive sample size sequential method was used according to Potvin et al. (2007) (Method B from paper):

- Bioequivalence was evaluated from the results in stage 1 using an α level of 2.94 %, regardless of the power achieved.

- If the bioequivalence criterion was met, then the study would have been stopped.
- If the bioequivalence criterion was not met, then the power at stage 1 with an α level of 2.94 % would have been evaluated.
- If stage 1 already had at least 80 % power, then the study would have been stopped.
- If power was <80 %, then the sample size would have been calculated based on the variance estimated at stage 1 and an α level of 2.94 % and the additional subjects required would have been randomized.

Following the completion of stage 1, it was determined that an additional four evaluable subjects were required to complete stage 2. This was based on the observed intra-subject CV from stage 1 of 18 % for $C_{\max}$. To allow for subject withdrawals, which was considered a greater possibility than in stage 1 due to the time of year and the greater possibility of subjects suffering from respiratory infections, an additional eight subjects were randomized into stage 2.

2.4 Study drug administration

All treatment administrations were performed in the Nuvisan GmbH Clinical Pharmacology Centre under direct medical supervision. The order of administration of the test product (azelastine hydrochloride nasal spray) and the reference product (Allergodil®) to each subject was determined by a randomization schedule generated by a computerized randomization generator. Each subject was allocated to receive both treatments in a randomized order, based on a Williams Latin Square design. A separate randomization list was generated for each stage of the study.

The study personnel involved in the blood sample analysis and pharmacokinetic parameter calculation were blinded from the randomization code during the whole study. The test and reference products were administered as a single dose in the morning of day 1 in two consecutive periods according to the crossover design. The dose administered was 0.28 mg (one nasal spray shot per nostril). There was a period of at least 14 days washout between the two study treatment periods. Prior to spraying each shot of the product into each nostril, the actuator was primed three times in a separate room to ensure accurate drug administration. In each study period, one spray of azelastine hydrochloride was administered into each nostril (two sprays in total) when subjects were in a sitting position with an upright posture of the head. Approximately 20 min before each product administration, subjects were asked to blow their nose gently with a tissue paper to clean it. The tip of the spray bottle was inserted approximately 0.5–1 cm into the nostril to deliver the drug. Two sprays of

the drug were administered within 1 min of each other. After the administration, subjects were requested not to blow their nose for a period of 60 min and, if possible, to refrain from sneezing. If subjects did blow their nose, they were withdrawn from the study. Furthermore, the subjects had to stay in an upright position for at least 2 h after drug administration.

2.5 Blood sample collection and drug analysis

Venous blood samples (7.5 mL) were collected with the subject in a sitting position from an indwelling catheter inserted in a forearm vein before drug administration and at 10 min $\pm$ 2 min, 20 min $\pm$ 2 min, 30 min $\pm$ 2 min, 1 h $\pm$ 10 min, 2 h $\pm$ 10 min, 3 h $\pm$ 10 min, 4 h $\pm$ 10 min, 6 h $\pm$ 10 min, 8 h $\pm$ 10 min, 12 h $\pm$ 30 min, 24 h $\pm$ 1 h, 48 h $\pm$ 1 h, 72 h $\pm$ 1 h, 96 h $\pm$ 1 h and 120 h $\pm$ 1 h after drug administration. 7.5 mL of blood was mixed with Li-heparin monovettes and stored on ice for no more than 20 min before centrifugation at 4 °C for 10 min at 2,500 $\times$ g to obtain plasma. Each plasma sample was immediately divided and transferred into two equal aliquots to standard polypropylene tubes and stored at -20 °C until analysis. The plasma concentrations of azelastine and its major metabolite desmethyl azelastine were determined at Nuvisan GmbH using a validated LC–MS/MS method in compliance with good laboratory practice regulations. The analytical method has a defined lowest level of quantification of 2.00 pg/mL and linear range from 2.00 pg/mL to 600.00 pg/mL for both azelastine and desmethyl azelastine.

2.6 Pharmacokinetic analysis

Pharmacokinetic analysis was conducted on the per-protocol (PP) population, defined as all subjects who satisfied all inclusion/exclusion criteria, completed the study, had evaluable data for both study periods and did not have any major protocol violation that may affect PK results. Subjects who withdrew from the study prior to completing both study periods were excluded. Pharmacokinetic parameters were calculated using Nuvisan GmbH validated SAS macro suite SAS-BA/BE (SAS version 8.2) using non-compartmental methods, e.g. linear trapezoidal rule for AUC, log-linear regression for λ_z , etc. (Cawello 1999). For the calculation of PK parameters, all concentration values below the lower quantification limit (BLQL) were set to zero. In case of missing concentration data at particular time points, no values were imputed. For calculations based on logarithms of values (e.g. terminal rate constant), BLQL values were disregarded. To reliably estimate $AUC_{0-\infty}$, at least three data points had to be present on the terminal log-linear phase of the plasma concentration

curve, otherwise the $AUC_{0-\infty}$ was not calculated. The value of $AUC_{0-\infty}$ was considered unreliable if the terminal area beyond the last quantified sample was greater than 20 % of the total $AUC_{0-\infty}$. $C_{\max}$ and $t_{\max}$ were regarded as unreliable if the maximal value was observed before or after a sample with missing data. In case of multiple peaks, $C_{\max}$ and $t_{\max}$ refer to the highest measured concentration even if there were earlier peaks. In case of two or more samples with the same concentration (as supplied by the analyst), $t_{\max}$ refers to the earlier of these.

The analysis of the primary variables was conducted according to a two-stage approach (Potvin et al. 2007). A linear fixed effects analysis of variance (ANOVA) model was used to analyse the logarithmically transformed (natural log) AUC_{0-t} and $C_{\max}$ using PROC GLM in SAS.

For stage 1, the model included factors for treatment, subject (sequence), period and sequence. For stage 2, the analysis included data from both stages and the effect of 'stage' was accounted for in the model. The full ANOVA model for stage 2 was specified with terms for sequence, stage, period (stage), treatment and subject (sequence $\times$ stage). The residual variance from the model was used to construct a two-sided 94.12 % CI for the difference between test and reference treatment. These were then back transformed (antilogged) to give point estimates and CI for the ratio of the treatment geometric means. For either stage, the criteria for bioequivalence for AUC_{0-t} and $C_{\max}$ were met if the 94.12 % CI for the treatment mean ratio was completely within the range 0.80–1.25. This method of conducting the analyses at adjusted significance levels (2.94 % at each stage) and with confidence levels adjusted appropriately preserves the overall type I error rate at 5 %. The intra-subject CV, calculated from the within subject residual mean square error from the ANOVA of the log-transformed data, was also presented as a measure of the within-subject variability. Subjects who withdrew from the study prior to completing both study periods were excluded from the statistical analysis. For descriptive statistics of plasma concentrations, BLQL values were replaced by half LLQ, except for BLQL values measured pre-dose, which were replaced by zero. Both primary variables were also summarized by treatment group using descriptive statistics. Mean plasma drug concentration/time curves were presented.

$t_{\max}$ was analysed non-parametrically by use of a series of Wilcoxon rank sum tests as described by Hills and Armitage (1979). The Hodges–Lehmann estimate of the median difference between treatments was presented with a corresponding 95 % CI according to the method described by Hodges and Lehmann (Hollander and Wolfe 1973). The secondary variables for AUC, $C_{\max}$ and $t_{\max}$ were summarized by treatment group using descriptive statistics.

2.7 Safety analysis

All subjects who received study treatment were included in the analysis and reporting of safety data. Adverse events (AEs) including severity and causality were listed by subject and summarized by treatment groups.

3 Results

Twenty-three subjects were enrolled in the study, 15 in stage 1 and 8 in stage 2, and were evaluable for safety. One subject experienced product leaking out of the nose during treatment administration, which was deemed as a major protocol violation, and was therefore excluded from the PP pharmacokinetic analysis.

The 23 subjects (13 males and 10 females) in the safety population had a mean (SD) age of 37.7 (11.3) years (range 21–55 years) and a mean (SD) BMI of 24.57 (2.64) kg/m². All subjects were white Caucasian. The 22 subjects (12 males and 10 females) included in the PP population PK analysis had a mean (SD) age of 37.4 (11.4) years (range 21–55 years) and a mean (SD) BMI of 24.42 (2.61) kg/m². The results of the study primary end points (AUC_{0-t} and $C_{\max}$) are summarized in Table 1. For both PK parameters, the 94.12 % CI for the ratio of geometric means (test/reference) was within the predefined bioequivalence range of 0.80–1.25. AUC_{0-t} and $C_{\max}$ were comparable between the two study treatments, indicating that the two formulations of azelastine hydrochloride were bioequivalent in terms of total exposure and peak exposure.

Mean azelastine PK profiles following single-dose administration of the new azelastine hydrochloride formulation and reference product Allergodil® are presented in Fig. 1. The results from the non-compartmental PK analysis showed a median $t_{\max}$ of 1 h (range: 0.2–8 h) for the new azelastine hydrochloride formulation and a median $t_{\max}$ of 2 h (range: 0.2–6 h) for Allergodil®. The difference in $t_{\max}$ between the two formulations is not statistically significant ($p = 0.4776$). The estimated geometric mean $AUC_{0-\infty}$ of the new azelastine hydrochloride formulation and Allergodil® was 1,748 and 1,621 h pg/mL, respectively. Summary statistics for other PK parameters, AUC_{0-t} , $AUC_{0-\infty}$, $C_{\max}$, and $t_{\max}$ for the metabolite desmethyl azelastine, are summarized in Table 2. The geometric means of $AUC_{(0-t)}$ ratios between desmethyl azelastine and azelastine for the two formulations were 0.54 for both. Mean plasma concentrations for desmethyl azelastine versus time profiles are presented in Fig. 2.

For the safety evaluation, vital signs, body weight, physical examinations and ECGs showed no clinically relevant changes during the study. An overall summary of AEs by treatment is presented in Table 3. No death and no

Table 1 AUC_{0-t} and C_{max} for azelastine for the two formulations (per protocol population)

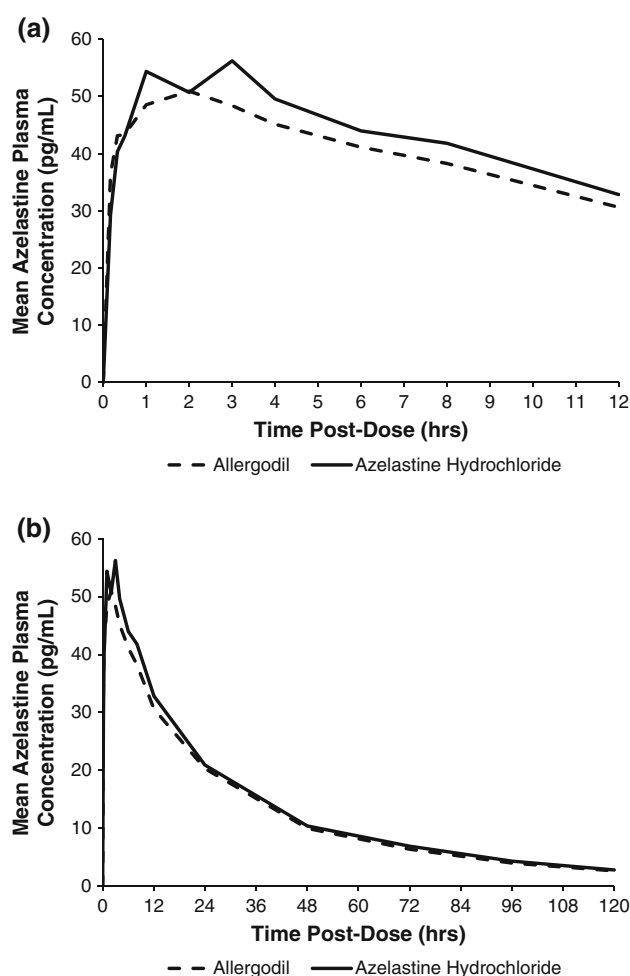
	Azelastine hydrochloride ($N = 22$)	Allergodil® ($N = 22$)	Ratio (94.12 % CI)
AUC_{0-t} (h pg/mL)	1,526.8	1,441.5	1.06 (0.99, 1.13)
C_{max} (pg/mL)	61.59	58.21	1.06 (0.95, 1.18)

Adjusted mean values calculated on log-transformed data are back transformed and presented for AUC_{0-t} and C_{max} in each treatment group. The ratio represents the ratio of geometric means of test and reference formulations

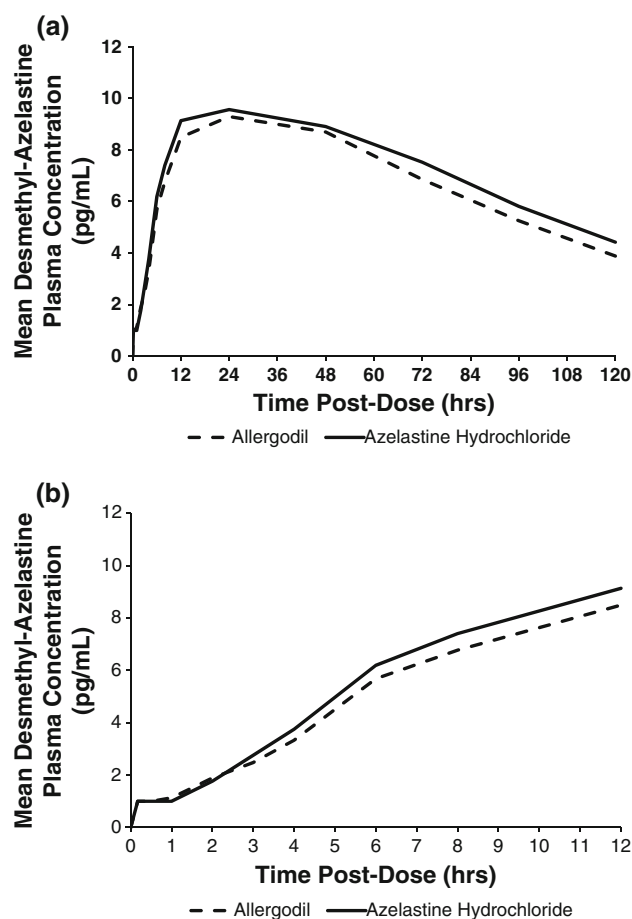
Table 2 PK parameters for desmethyl azelastine for the two formulations (per protocol population)

	Azelastine hydrochloride ($N = 22$)	Allergodil® ($N = 22$)
$AUC_{(0-t)}$ (h pg/mL)	827.8 (462, 1,423)	779.0 (437, 1,404)
$AUC_{(0-\infty)}$ (h pg/mL)	1,327.2 (741, 2,232)	1,097.7 (684, 2,033)
C_{max} (pg/mL)	9.76 (4.6, 17.9)	9.52 (4.2, 18.0)
t_{max} (h)	36.0 (12, 96)	48.0 (12, 96)

Geometric mean values (range) are presented for AUC parameters and C_{max} . Median values (range) are presented for t_{max}

**Fig. 1** Mean azelastine plasma concentration over time for the two formulations (per protocol population): **a** from time 0 to 120 h after dosing; **b** from time 0 to 12 h after dosing

serious AEs were reported. In total, six subjects (26.1 %) reported ten AEs: four subjects from the new azelastine hydrochloride formulation group (17.4 %) reported four AEs and three subjects from the Allergodil® group

**Fig. 2** Mean desmethyl azelastine plasma concentration over time for the two formulations (per protocol population): **a** from time 0 to 120 h after dosing; **b** from time 0 to 12 h after dosing

(13.0 %) reported six AEs. All AEs were of mild intensity. Two treatment-related AEs occurred in the new azelastine hydrochloride formulation group and three treatment-related AEs occurred in the Allergodil® group. All subjects had normal laboratory values or slightly abnormal ones which were judged as not clinically significant by the study investigator, except one subject who showed abnormal

Table 3 Number of subjects with treatment-emergent AEs by treatment and system organ class

System organ class	Adverse events	Azelastine hydrochloride (<i>N</i> = 23) <i>n</i> (%) nAE	Allergodil® (<i>N</i> = 23) <i>n</i> (%) nAE	Overall (<i>N</i> = 23) <i>n</i> (%) nAE
Number of subjects with AEs		4 (17.4) 4	3 (13.0) 6	6 (26.1) 10
Nervous system disorders	Overall	2 (8.7) 2	2 (8.7) 2	4 (17.4) 4
	Headache	1 (4.3) 1	1 (4.3) 1	2 (8.7) 2
	Dizziness	1 (4.3) 1	0	1 (4.3) 1
	Dysgeusia	0	1 (4.3) 1	1 (4.3) 1
Respiratory, thoracic and mediastinal disorders	Overall	1 (4.3) 1	2 (8.7) 2	3 (13.0) 3
	Epistaxis	0	1 (4.3) 1	1 (4.3) 1
	Nasal discomfort	1 (4.3) 1	0	1 (4.3) 1
	Rhinorrhoea	0	1 (4.3) 1	1 (4.3) 1
Investigations	Overall	0	1 (4.3) 2	1 (4.3) 2
	Bacterial test pos.	0	1 (4.3) 1	1 (4.3) 1
	Protein urine	0	1 (4.3) 1	1 (4.3) 1
General disorders and administration site conditions	Overall	1 (4.3) 1	0	1 (4.3) 1
	Fatigue	1 (4.3) 1	0	1 (4.3) 1

n (%) represents the number (percent) of subjects with AEs. nAE represents total number of adverse events

values in urinalysis at discharge, which returned to normal at a follow-up examination 2 days later.

4 Discussion

This study demonstrated bioequivalence between the new azelastine hydrochloride nasal spray formulation and the marketed reference Allergodil® after single-dose administration. The 94.12 % CI for the ratio of geometric means (test/reference) was within the predefined bioequivalence range of 0.80–1.25, indicating that the extent of absorption (AUC_{0-t}) and peak exposure (C_{max}) between the two treatments were equivalent. There was no statistically significant difference in t_{max} between the two treatments, even though the broad range of t_{max} values indicated that there was a high inter-individual variability. The PK profiles of the major metabolite, desmethyl azelastine, were also similar for the two azelastine hydrochloride formulations.

The new azelastine hydrochloride formulation and Allergodil® were well tolerated following a single dose of nasal administration of 0.28 mg. Both formulations did not show any clinically significant effects on blood pressure, heart rate, ECG, blood and urine laboratory results and physical examination. No serious AEs were reported during the study. Few mild AEs occurred in this study, which were consistent with the well-known safety profile of the marketed reference product.

The relevance of the PK comparability between the two formulations demonstrated in this study to local treatment of allergic rhinitis should also be discussed. The intention of using local nasal spray of azelastine is

to minimize the systemic exposure to the drug. Therefore, the nasal spray will have less systemic side effects while exerting an anti-inflammatory effect on the nasal mucous membrane. The current study demonstrated that the systemic exposure and C_{max} of the two nasal spray formulations were similar, which was supported by the similar adverse event profiles between the two formulations. However, the azelastine concentrations in the local nasal tissues from the two formulations were not measured due to technical difficulties. The similarity of PK profiles between the two formulations demonstrated in this study suggests the bioequivalence of these two nasal formulations. The ultimate proof of clinical similarity in safety and efficacy for the two formulations can be obtained by side by side comparison in a clinical efficacy study.

Acknowledgments The authors appreciate the excellent help in preparing the manuscript and support of the study by Renata Perego, Elena Tedeschi and Sharron Ashburner. We also thank Dr. Nick P. Kronfield and Mairead North for their critical discussion of the study design and manuscript review. This study was sponsored by Glaxo-SmithKline Consumer Healthcare.

References

- Bousquet J, Khaltaev N, Cruz AA et al (2008) Allergic rhinitis and its impact on asthma (ARIA) 2008 update (in collaboration with the World Health Organization, GA(2)LEN and AllerGen). *Allergy* 63:8–160
- Casale TB (1989) The interaction of azelastine with human lung histamine H_1 , beta, and muscarinic receptor binding sites. *J Allergy Clin Immunol* 83:771–776
- Cawello W (1999) Parameters for compartment-free pharmacokinetics. Shaker, Aachen

- Chand N, Pillar J, Nolan K, Diamantis W, Sofia RD (1989) Inhibition of allergic and nonallergic leukotriene C4 formation and histamine secretion by azelastine: implications for its mechanism of action. *Int Arch Allergy Appl Immunol* 90:67–70
- Ciprandi G, Pronzato C, Passalacqua G et al (1996) Topical azelastine reduces eosinophil activation and intercellular adhesion molecule-I expression on nasal epithelial cells: an anti-allergic activity. *J Allergy Clin Immunol* 98:1088–1096
- Hills M, Armitage P (1979) The two period cross-over clinical trial. *Br J Clin Pharmacol* 8:7–20
- Hollander M, Wolfe DA (1973) Nonparametric statistical methods. Wiley, New York
- Ito H, Nakamura Y, Takagi S, Sakai K (1998) Effects of azelastine on the level of serum interleukin-4 and soluble CD23 antigen in the treatment of nasal allergy. *Arzneimittelforschung* 48:1143–1147
- Nieber K, Baumgarten C, Rath sack R, Furkert J, Laake E, Müller S, Kunkel G (1993) Effect of azelastine on substance P content in bronchoalveolar and nasal lavage fluids of patients with allergic asthma. *Clin Exp Allergy* 23:69–71
- Potvin D, DiLiberti CE, Hauck WW, Parr AF, Schuirmann DJ, Smith RA (2007) Sequential design approaches for bioequivalence studies with crossover designs. Wiley InterScience (www.interscience.wiley.com), Pharmaceutical statistics. doi: 10.1002/pst.294
- Shinoda M, Watanabe N, Suko T, Mogi G, Takeyama M (1997) Effects of anti-allergic drugs on substance P (SP) and vasoactive intestinal peptide (VIP) in nasal secretions. *Am J Rhinol* 11:237–241
- Wallace DV, Dykewicz MS, Bernstein DI et al (2008) Diagnosis and management of rhinitis: an updated practice parameter. *J Allergy Clin Immunol* 122:S1–S84
- Zechel HJ, Brock N, Lenke D, Achterrath-Tuckermann U (1981) Pharmacological and toxicological properties of azelastine, a novel anti-allergic agent. *Arzneimittelforschung* 31:1184–1193