Clinical signs: No drug-related findings were noted

Body weights: No drug-related findings were noted

Gross pathology: No drug-related findings were noted

Organ weights: No drug-related findings were noted

Histopathology: Addition of sucralose to the azelastine-containing solution did not increase in the incidence of microscopic changes in the respiratory system in rats. Incidences of inflammation (incidence: 3 – 6/10) and mononuclear/lymphocyte infiltration (incidence: 2 – 5/10) were observed in all groups in both sexes. There was no discernable dose-response relationship in the incidence of these abnormalities and sucralose concentration in either of the sexes. The results indicate that sucralose does not adversely affect the respiratory tract in rats in addition to that induced by azelastine under the conditions tested.

Study Title: 14-Day Intranasal Toxicity Study with Azelastine and Sucralose in Beagle Dogs (Study 0437RM57.003)

Key findings: Intranasal administration of sucralose at concentrations up to 0.15% does not significantly affect the local toxicity of azelastine in dogs. Beagle dogs (3/sex/group) were treated with an azelastine-containing vehicle (reference) in the absence of sucralose for 14 days. The reference (Group 1) consisted of 0.1% azelastine, —% hypromellose ——— % edetate disodium, —% sorbitol, — % sodium citrate, and — % benzalkonium chloride. Formulations for groups 2-4 contained the components listed above and different sucralose concentrations (0.05%, 0.1% and 0.15% for Groups 2, 3 and 4, respectively). Each nostril was instilled with 0.1 ml solution twice a day which corresponded to azelastine/sucralose doses of 1.8/0, 1.8/0.9, 1.8/1.8 and 1.8/2.7 μg/cm^2 based on nasal surface area and 40/0, 40/20, 40/40 and 40/60 μg/kg based on body weight. The respiratory system was examined microscopically. Low incidences of abnormalities were observed in the reference group in both sexes. Addition of sucralose up to 0.15% did not significantly increase the incidence of these abnormalities. The sucralose NOAEL for local toxicity was 60 μg/kg/day and 2.7 μg/cm^2/day based on body weight and nasal surface area comparisons, respectively. The NOAEL for azelastine was not established.

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Study number:	0437RM57.003
Volume #, and page #:	Vol. C7.2, p 1 in IND 69785
Report Date:	February 3, 2006
Conducting laboratories and location:	———
Date of study initiation:	September 19, 2005
study completion date:	October 7, 2005
GLP compliance:	Yes, with a signed page
QA reports:	Yes, with a signed page
Drug, lot #, radio-label, and %	Batches 03-32-02C, 03-37-01C, 03-33-02C and

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purity: Lot 0000002635 (Astelin®, 0.1% azelastine HCl), Purity: 99.6%.

Methods:

Beagle dogs (3/sex/group) were treated with a vehicle (reference) containing 0.1% azelastine in the absence or presence of sucralose for 14 days. The reference (Group 1) consisted of 0.1% azelastine, [redacted] % hypromellose [redacted] % edetate disodium, [redacted] % sorbitol, [redacted] % sodium citrate, and [redacted] % benzalkonium chloride. Sucralose concentrations for the treated groups were 0.05%, 0.1% and 0.15% for Groups 2, 3 and 4, respectively. Each nostril was instilled with 0.1 ml solution twice a day. The total azelastine dose was 1.8 μg/cm^2 based on nasal surface area or 40 μg/kg based on body weight in each group including the reference group. The respective total daily dose of sucralose in the low, mid and high-dose groups was 0.9, 1.8 and 2.7 μg/cm^2 on a nasal surface area basis and 20, 40 and 60 μg/kg on a body weight basis.

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Species/strain:	Dogs /Beagle
#/sex/group (main study):	3
Age at commence of expose:	Approximately 5 – 7 months
Weight:	5.2 – 8.6 kg
House:	Individually housed
Route, form:	Nasal instillation, 0.1 ml/nostril, 2x per day
Treatment duration:	Twice a day for 14 days, 6 hours between doses on the same day
Reference:	0.1% azelastine with other excipients

Doses in administered units:

Groups	1	2	3	4
Treatment	Reference	Reference + 0.05% sucralose	Reference + 0.10% sucralose	Reference + 0.15% sucralose
Sucralose /azelastine dose [a]				
mg/kg (based on body weight)	0/1.8	0.9/1.8	1.8/1.8	2.7/1.8
μg/cm^2 (based on a nasal surface area)	0/40	20/40	40/40	60/40
mg/ml (based on concentration)	0/1.0	0.5/1.0	1.0/1.0	1.5/1.0

a. The doses were calculated from the following parameters: daily instillation volume of 0.4 ml (0.1 ml/nostril, twice a day), nasal surface area of 220 cm^2/dog, body weight of 10.0 kg/dog, azelastine concentration of 1 mg/ml and sucralose concentrations of 0.5, 1 and 1.5 mg/ml.

Observations and times:

Clinical signs:	Once daily
Body Weight:	Weekly
Food consumption:	Not assessed
Ophthalmoscopy	Not assessed
EKG:	Not assessed

Hematology:	Not assessed
Clinical chemistry:	Not assessed
Urinalysis:	Not assessed
Pathology:	End of treatment (24 hrs after the last treatment)
Organ weights:	Adrenal glands, brain, heart, kidneys, liver, lungs with trachea, gonads, pancreas, pituitary gland, prostate, spleen, and thymus, thyroid/parathyroid, and uterus
Histology:	Respiratory system only: nasal cavity, nasopharynx, larynx, trachea, lung with mainstem bronchus, tracheobronchial lymph nodes. Adequate Battery: yes (x), no ( )— as agreed during the May 8, 2005 End-of-Phase 2 meeting. The minutes state: "The studies should be designed to adequately evaluate the toxicity profile of sucralose and the sweetened formulation in the respiratory tract." Peer review: yes ( ), no (x)

Results:

Mortality: None

Clinical signs: No drug-related findings were noted

Body weights: No drug-related findings were noted

Gross pathology: No drug-related findings were noted

Organ weights: No drug-related findings were noted

Histopathology: Addition of sucralose to the azelastine-containing solution did not increase in the incidence of microscopic changes in the respiratory system in dogs. Low incidences of abnormalities were observed in the azelastine formulation reference group in both sexes. Addition of sucralose did not increase significantly the incidence of these abnormalities in any of the treated groups. The results indicate that sucralose does not adversely affect the respiratory tract in dogs at the conditions tested.

Study Title: 14-Day Nasal Irritation Procedure in Rats (Study 16365)

Key findings: Intranasal administration of sucralose may enhance the irritating potential of azelastine in the nasal cavity in rats. Sprague-Dawley rats (10/sex/group) were treated with Astelin® Nasal Spray solution (Group 3, 0.1% azelastine), a vehicle (Group 2; the vehicle consisted of —% hypromellose ——— % edetate disodium, and — % benzalkonium chloride), the vehicle plus 0.15% sucralose (Group 1), or the vehicle plus 0.15% sucralose and 0.1% azelastine (Group 4), or vehicle plus 0.15% sucralose and 0.15% azelastine (Group 5) for 14 days. Each solution (0.1 ml) was instilled to each nostril twice a day. The respective total daily doses of

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azelastine/sucralose for groups 1, 2, 3, 4 and 5 were 60/0, 0/0, 0/40 and 60/40 and 60/60 μg/cm^2 on a nasal surface area basis, and 2.4/0, 0/0. 0/1.6, 2.4/1.6, 2.4/2.4 on a body weight basis. Rats treated with the vehicle or vehicle plus sucralose (Groups 1 and 2) showed no discernable lesions in the nasal cavity. Rats treated with sucralose in the presence of azelastine (Groups 4 and 5) or those treated with Astelin (group 3) showed microscopic changes in nasal cavity. The changes include hemorrhage (focal or multi-focal), inflammation and hyaline droplets in the respiratory epithelium region and hypertrophy/hyperplasia of the goblet cells. The addition of sucralose appeared to result in increases in goblet cell hyperplasia while the 0.15% azelastine formulation containing sucralose increased the incidence of hemorrhage. The respective incidence (males and females combined due to lack of gender difference) for Groups 1, 2, 3, 4 and 5 was 0/20, 0/20, 1/20, 0/20 and 4/20 for hemorrhage and 0/20, 0/20, 2/20, 8/20 and 8/20 for goblet cell hypertrophy or hyperplasia. The NOAEL for sucralose alone was 0.15% (or 60 μg/cm^2 on a nasal surface area basis). The NOAEL for azelastine was not identified, neither was that for azelastine and sucralose in combination.

Study number:	16365
Volume #, and page #:	Vol. 2.1, p 68 and 3.1, p 3 (amended report) in IND 69,785
Report Date:	May 27, 2005 (Amended report)
Conducting laboratories and location:	[redacted]
Date of study initiation:	November 1, 2004
study completion date:	February 27, 2005
GLP compliance:	Yes, with a signed page
QA reports:	Yes, with a signed page
Drug, lot #, radio-label, and % purity:	Batch 03-36-01c (with 0.15% sucralose), Purity not available. , This is not a major deficiency because the study is not pivotal .

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Formulation/vehicle[a]:

Group	3	2	1	4	5
Description	Astelin®	Vehicle	Vehicle plus sucralose	Vehicle plus 0.1% azelastine & 0.15% sucralose	Vehicle plus 0.15% azelastine & 0.15% sucralose
Azelastine HCl (%)	0.100	-	-	0.100	0.150
Sucralose (%)	-	-	0.15	0.15	0.15
Citric acid (%) [b]	[redacted]	-	-	-	-
Dibasic sodium phosphate (%)	[redacted]	-	-	-	-
[redacted] % sorbitol [redacted] (%)	-	[redacted]	[redacted]	[redacted]	[redacted]

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a. Each formulation also contains [redacted] % hypromellose [redacted] % edentate disodium, [redacted] % benzalkonium chloride and purified water (Q.S.).

b. [redacted]

Methods:

Sprague-Dawley rats (10/sex/group) were treated for 14 days with one of the following: Astelin® Nasal Spray solution[1] (Group 3, containing 0.1% azelastine), a vehicle (Group 2), the vehicle plus 0.15% sucralose (Group 1), or the vehicle plus 0.15% sucralose and 0.1% azelastine (Group 4), or the vehicle plus 0.15% sucralose and 0.15% azelastine (Group 5). The vehicle consisted of % hypromellose % edentate disodium, and % benzalkonium chloride. Each solution (0.1 ml) was instilled to each nostril twice a day. The respective total daily doses of azelastine/sucralose for groups 1, 2, 3, 4, and 5 were 60/0, 0/0, 0/40 and 60/40 and 60/60 μg/cm^2 on a nasal surface area basis, and 2.4/0, 0/0. 0/1.6, 2.4/1.6, 2.4/2.4 on a body weight basis.

Doses:

Groups	1	2	3	4	5
Treatment	Vehicle + 0.15% sucralose	Vehicle	Astelin®	Vehicle + 0.15% sucralose + 0.1% azelastine	Vehicle + sucralose + 0.15% azelastine
Sucralose /azelastine dose [a]					
μg/cm^2 (based on a nasal surface area)	60/0	0/0	0/40	60/40	60/60
mg/kg (based on body weight)	2.4/0	0/0	0/1.6	2.4/1.6	2.4/2.4
Mg/ml (based on concentration)	1.5/0	0/0	0/1.0	1.5/1.0	1.5/1.5

a. The doses were calculated from the following parameters: daily instillation volume of 0.4 ml (0.1 ml/nostril, twice a day), nasal surface area of 10 cm^2/rat, body weight of 0.25 kg/rat, azelastine concentration of 1 mg/ml and sucralose concentrations of 0.5, 1 and 0.15 mg/ml.

Species/strain:	Rats / :CD(SD)
#/sex/group (main study):	10
Age:	Approximately 9 weeks
Weight:	M: 262 - 312 g; F: 197 – 232 g
Route, formulation, volume and infusion rate:	Nasal instillation, solution, 1 ml/nostril, twice daily, 6 hrs between doses
Sampling times:	See below
Unique study design:	NA

Observations and times:

Clinical signs:	Once daily
Body Weights:	Weekly
Food consumption:	Not assessed
Ophthalmoscopy	Not assessed
EKG:	Not assessed
Hematology:	Not assessed
Clinical chemistry:	Not assessed
Urinalysis:	Not assessed

[1] Astelin® Nasal Spray consists of 0.1 azelastine HCl, hypromellose % edentate disodium, % benzalkonium chloride, % citric acid, % dibasic sodium phosphate and % sodium citrate. The last 3 ingredients were absent in the vehicle of the current study.

Gross Pathology: End of treatment (24 hrs after the last treatment)
Organ weights: Adrenal glands, brain, heart, kidneys, liver, lungs with trachea, gonads, pancreas, pituitary gland, prostate, spleen, and thymus, thyroid/parathyroid, uterus
Histology: Respiratory system only: nasal cavity, nasopharynx, larynx, trachea, lung with mainstem bronchus, tracheobronchial lymph nodes.
Adequate Battery: yes (x), no ( )— as agreed during the May 8, 2005 End-of-Phase 2 meeting.
Peer review: yes ( ), no (x)

Results:

Mortality: None

Clinical signs: No drug-related findings were noted.

Body weights: No drug-related findings were noted.

Gross pathology: No drug-related findings were noted.

Organ weights: No drug-related findings were noted.

Histopathology: Rats treated with 0.15% sucralose in the presence of azelastine showed increases in the incidence of lesions in the nasal cavity (Table 2) compared to the other three groups, although no treatment-related effect was observed at 0.15% sucralose in the absence of azelastine. The lesions include inflammation, hyaline droplets in the respiratory epithelium region, hemorrhage and hypertrophy/hyperplasia of the goblet cells. Of note, the approved Astelin formulation produced many of the same findings and at similar incidence. The addition of sucralose appeared to result in increases in goblet cell hyperplasia while the 0.15% azelastine formulation containing sucralose increased the incidence of hemorrhage. There were no apparent differences in the remaining parameters among the groups.

Table 2. Notable Findings in Nasal Turbinates

Findings	Sex	Treatment (n = 10/group)				
		Vehicle Plus 0.15% sucralose	Vehicle	Astelin®	Vehicle plus sucralose & 0.1% azelastine	Vehicle plus sucralose & 0.15% azelastine
Hemorrhage, focal-multifocal	M	0	0	1	0	2
	F	0	0	0	0	2
Respiratory epithelium: hyaline droplets	M	0	0	9	9	10
	F	0	0	10	10	10
Necrosis, individual cell,	M	0	0	2	0	0
Inflammation/acute, multi-focal	M	0	0	7	10	10
	F	0	0	10	10	9
Goblet cell: hypertrophy/hyperplasia	M	0	0	2	3	5
	F	0	0	0	5	3

2.6.6.9 Discussion and Conclusions

The irritation/toxicity potential of sucralose and the new azelastine formulation to the respiratory tract was evaluated in two 14-day intranasal toxicity studies in rats and dogs (Studies 0437RM57.002 and 003). A volume (0.1 ml) of the testing formulation was instilled into the nasal cavity twice a day for 14 days in each species. Sample sizes were 10 and 3/sex/dose in rats and dogs, respectively. Sucralose concentrations were 0, 0.05%, 0.1% and 0.15% respectively. The total daily doses of azelastine/sucralose for the control, low, mid and high dose groups correspond to 40/0, 40/20, 40/40 and 40/60 μg/cm^2 in rats and 1.8/0, 1.8/0.9, 1.8/1.8 and 1.8/2.7 μg/cm^2 in dogs, based on nasal surface area. The reference solution contained 0.1% azelastine, ~1% hypromellose ~ ——— % edentate disodium, ~% sorbitol, —— % sodium citrate, and —— % benzalkonium chloride. Results showed that sucralose in the azelastine formulation at concentrations up to 0.15% was not additionally irritating to the respiratory tract in either rats or dogs compared to the reference groups. The respective sucralose NOAELs were 60 and 2.7 μg/cm^2 in rats and dogs on the nasal surface area basis, and 2,400 and 60 μg/kg/day on a body weight basis. The NOAEL for azelastine was not established in rats or dogs, but the studies were not designed to do so.

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Another 14-day intranasal toxicity study with a different formulation was completed in rats by a different laboratory (Study 16365). This study showed that: 1) neither sucralose nor a vehicle without sucralose or azelastine was irritating to the nasal cavity, 2) Astelin®, the marketed formulation, was irritating to the nasal cavity, 3) sucralose appeared to enhance the irritation potential of azelastine, and 4) a formulation with 0.15% azelastine and sucralose induced an increased incidence of hemorrhage in the nasal cavity. The NOAEL for sucralose alone was 1.5 mg/ml based on concentration or 60 μg/cm^2 based on the nasal surface area respectively. The NOAEL for azelastine was not identified, neither was it identified for azelastine in combination with sucralose. This study is not used for safety evaluation because it employed a not to-be-developed formulation though it provides information related to the local irritation potential of the approved Astelin formulation.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Summary:

The irritation/toxicity potential of sucralose to the respiratory tract was evaluated in two 14-day intranasal toxicity studies in rats and dogs (Studies 0437RM57.002 and 003). The studies were done with 0.1% azelastine and other excipients proposed for clinical use in the absence or presence of sucralose. Sucralose concentrations were 0, 0.05%, 0.1% and 0.15% respectively for the control, low, mid and high dose, respectively. The testing formulation (MP33-03) was instilled intra-nasally with 0.1 ml/nostril twice a day for 14 days to Sprague-Dawley rats (10/sex/group) and beagle dogs (3/sex/group). The control solution contained

0.1% azelastine, ′— % hypromellose ′ ——— % edentate disodium, —% sorbitol, ′ —,% sodium citrate, and — % benzalkonium chloride. The respective total daily doses of azelastine/sucralose of the control, low, mid and high-dose groups, based on nasal surface area, were 40/0, 40/20, 40/40 and 40/60 μg/cm^2 in rats and 1.8/0, 0.9/1.8, 1.8/1.8 and 1.8/2.7 in dogs. Results showed no increases in the incidence of microscopic abnormalities in the sucralose-treated groups over the control. These studies indicated that sucralose at concentrations up to 0.15% is not additionally irritating to the respiratory tract in either rats or dogs compared to the azelastine formulation without sucralose. The sucralose NOAELs was 60 and 2.7 μg/cm^2 in rats and dogs, respectively based on the nasal surface area. The NOAEL for azelastine was not established in either rats or dogs. Note these studies were not designed to identify NOAELs. These toxicity studies did not reveal any safety concerns regarding to the proposed nasal use of sucralose or the proposed reformulation of the 0.1% azelastine formulation.

The NOAELs for sucralose in rats and dogs provide a reasonable safety margin for the proposed use of the excipients in humans. The nonclinical safety evaluation of the current clinical protocol concentrates on the local reactions only. This is because there is no safety concern about systemic toxicity of the proposed use of either azelastine or sucralose. The sponsor proposes to give each patient up to 2 actuations of MP03-33/nostril twice a day. Each actuation delivers 137 and — μg of azelastine and sucralose. The total daily dose of each component is 548 and μg of azelastine and sucralose, respectively. They correspond to total daily dose of — and ′— mg/cm^2 for azelastine and sucralose, based on a nasal surface area of 180 cm^2 in an adult patient. b(4)

There is no nonclinical safety concern about the proposed use of azelastine. The proposed use of 0.1% azelastine (i.e., volume and concentration per actuation and frequency/day) is identical to that described approved labeling of the currently marketed Astelin® Nasal Spray. The safety of azelastine has been established by the available nonclinical and clinical data. The newly completed 2-week intranasal toxicity in rats and dogs did not reveal any new toxicity. The proposed use of 0.1% azelastine is considered safe.

Similarly, there is no safety concern about the proposed use of sucralose. As previously indicated, the NOAEL for sucralose is 60 and 2.7 μg/cm^2 in rats and dogs, based on nasal surface area. The rat data provides a safety margin of 13. The NOAEL value in dogs is similar to that of the expected human exposure. Toxicity of sucralose has been well characterized and the systemic toxicity is not of safety concern. The local toxicity is determined by the concentration, the volume of administration and bioavailability etc. The concentration is probably the predominant factor in the nasal toxicity of sucralose. The sponsor has tested the proposed clinical formulations at apparently the maximum feasible doses. The testing did not reveal signals for safety concern about the local toxicity of sucralose. The proposed use of sucralose is of no safety concern. Overall, the completed toxicity studies in rats and dogs are deemed adequate to support the safety of clinical protocol MP430.

Table 3 Safety Margins for the Proposed Use of Sucralose and Azelastine

Parameter	Compound	Units	Animal NOAEL		Human (Proposed Use) [a]	
			Rat	Dog	Low Dose	High Dose
Exposure	Sucralose	$\mu g/cm^2$	60	2.7	1.7	3.3
	Azelastine	$\mu g/cm^2$	-	-	-	-
Safety Margin	Sucralose	N/A	N/A	N/A	55	27
	Azelastine	N/A	N/A	N/A	-	-

a. Based on 1 or 2 actuations/nostril, twice a day in an adult patient with a nasal surface area of 180 cm^2.
b. N/A, not applicable; -, not established.

Internal recommendations

The February 13, 2006, submission (Serial No. 015) has adequately addressed the nonclinical-hold issue identified in a pharmacology and toxicology review by Dr. Luqi Pei with the completion date of November 4, 2005 during the nonclinical safety evaluation of the clinical Protocol MP430 submitted on September 21, 2005. Submission 015 also fulfills MedPointe's nonclinical commitment to submit two 2-week intranasal toxicity studies prior to the initiation of Study MP430 as stated in the Division's action letter dated November 4, 2005. Currently, there are no outstanding nonclinical issues concerning the safety of the proposed clinical protocol. It is recommended that Protocol MP430, as amended in the March 3, 2006 submission (Serial No. 016), be allowed to proceed.

External Recommendation:

None.

Luqi Pei, Ph.D.
Senior Pharmacologist/toxicologist