Local and Systemic Toxicity of Intraoral Submucosal Injections of Phentolamine Mesylate (OraVerse)

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OraVerse™, an injectable formulation of phentolamine mesylate (PM), was recently approved by the U.S. Food and Drug Administration (FDA) for reversal of anesthesia of the lip and tongue and associated functional deficits resulting from an intraoral submucosal injection of a local anesthetic containing a vasoconstrictor. Because PM had not been approved previously for submucosal administration, 2 Good Laboratory Practices (GLP) studies in dogs designed to investigate systemic toxicity and the local effects of single and repeated dosing of OraVerse on the inferior alveolar nerve and branches of the superior alveolar nerve and adjacent soft tissues after local administration were conducted. Systemic toxicity was measured by preinjection and postinjection clinical examinations, clinical chemistry, and gross and microscopic examinations of major organs after necropsy. No evidence of systemic toxicity was detected. Local nerve and adjacent tissue damage was assessed by conventional histopathology. Nerve degeneration was evident in 1 animal. Mild perineural inflammation adjacent to the inferior alveolar nerve and inflammatory exudates were observed in submucosal tissues in several animals. No changes were observed in the nerves at injection sites of dogs from any dose group that were considered directly related to the test articles. These data reveal that single and repeated intraoral administrations of OraVerse are well tolerated in beagle dogs.

Key Words: Anesthesia reversal; Local and systemic toxicity.

INTRODUCTION

Local anesthetic solutions with or without vasoconstrictors that are available for use in clinical dentistry produce local anesthesia of teeth and surrounding soft tissues of varying duration.¹ Typically, teeth recover more quickly than surrounding bone and soft tissues such as lips and tongue. This soft tissue anesthesia lasts approximately 3 to 5 hours² and frequently is substantially longer than necessary for completion of many routine dental procedures. Patients report that

this lingering anesthesia interferes with normal activities such as speaking, drinking, eating, and avoiding drooling.³ Many have the perception of an altered appearance and would prefer to avoid prolonged perioral anesthesia.

OraVerse is a new proprietary, stabilized, injectable liquid formulation of phentolamine mesylate (PM). PM, used since 1952 in several medical indications, is a nonspecific α -adrenergic inhibitor, an effect of which is vasodilation. Following local anesthetic with a vasoconstrictor and a dental or periodontal maintenance procedure, local submucosal injections of OraVerse accelerated recovery from anesthesia of the lips and tongue in children (6 to 11 years old),⁴ adolescents, and adults, and return of normal function in adolescents and adults by approximately 50% when compared with individuals who received sham injections.⁵

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Table 1. Study Design, Single-Dose Study*

A					
Group	Treatment	Dose, $\mu\text{g}/\text{kg}$	Day of Necropsy		
			2	4	15
1	Control (vehicle)	0	3	2	2
2	Phentolamine mesylate	12	3	2	2
	HTAEDA	0.27			
	Phentolamide HCl	0.20			
3	Phentolamine mesylate	120	3	2	2
	HTAEDA	2.7			
	Phentolamide HCl	2.0			
4	Phentolamine mesylate	120	3	2	2
	HTAEDA	0			
	Phentolamide HCl	0			
5	Phentolamine mesylate	0	3	2	2
	HTAEDA	2.7			
	Phentolamide HCl	2.0			

* n = 7/group.

B				
Group	Treatment	Dose, $\mu\text{g}/\text{kg}$	Dosing Solution Concentration, $\mu\text{g}/\text{mL}$	Total Number of Animals
1	Control (vehicle)	0	0	3
2	Phentolamine mesylate	12	235	3
	HTAEDA	0.27	5.25	
	Phentolamide HCl	0.20	3.9	
3	Phentolamine mesylate	120	2350	3
	HTAEDA	2.7	52.5	
	Phentolamide HC	2.0	39.0	
4	Phentolamine mesylate	120	2350	3
	HTAEDA	0	0	
	Phentolamide HC	0	0	
5	Phentolamine mesylate	0	0	3
	HTAEDA	2.7	52.5	
	Phentolamide HC	2.0	39.0	

* n = 3/group.

OraVerse contains 0.4 mg PM in 1.7 mL. Two impurities in OraVerse are formed as a result of degradation of PM (m-[N-(2-imidazolin-2-yl-methyl)-p-toluidino]-phenol monomethanesulfonate) during storage. One of these results from hydrolysis of the imidazole ring, creating a species designated as “HTAEDA”; N¹-2-[N-(3-hydroxyphenyl)-N-(4-toluy)aminoacetyl]ethylene-diamine (C₁₇H₂₁N₃O₂). The second results from oxidation of the methylene bridging the central nitrogen atom of phentolamine and the imidazole ring, resulting in a species designated as “phentolamide”; N-(3-hydroxyphenyl)-N-(4-methylphenyl)-2-imidazolyl carboxamidehydrochloride (C₁₇H₁₈ClN₃O₂ * HCl). To the best of our knowledge, this species has not been reported in the chemical literature. It is not listed in the United States Pharmacopeia or the European Pharmacopeia.

Results from the single-dose local tolerance study revealed no systemic or local toxicity resulting from

an oral submucosal injection of PM and/or mixtures of HTAEDA and phentolamide. Because clinical use of OraVerse may involve multiple injections, a repeated-dose systemic and local tolerance study in beagle dogs designed to study the effects of multiple injections was conducted. This experiment tested the null hypothesis that systemic and local tolerance to single or multiple, sequential submucosal injections of PM, phentolamide, and HTAEDA does not differ from that seen in controls. The data revealed no significant systemic or local toxicity of these compounds and hence failed to support rejection of the null hypothesis.

METHODS

These studies were conducted at Northern Biomedical Research Inc, Muskegon, Mich, in accordance with the

Table 2. Tissue List

Adrenals	Heart	Pituitary	Stomach
Aorta	Ileum	Prostate	Testes
Brain	Inferior alveolar nerve	Rectum	Thyroids (with parathyroids)
Cecum	Injection sites (mandibular, maxillary)*	Rib with marrow	Thymus
Colon	Jejunum	Salivary gland	Tongue
Duodenum	Kidneys	Sciatic nerve	Trachea
Esophagus	Liver (with gallbladder)	Skeletal muscle	Trigeminal ganglion
Eyes with optic nerves	Lungs with bronchi	Skin (with mammary gland)	Ureter
Epididymides	Lymph nodes (mandibular/mesenteric/mediastinal)	Spinal cord (cervical, thoracic, and lumbar)	Urethra
Femur with joint	Pancreas	Spleen	Urinary bladder
Gross lesions			

* Gingival/mucosal tissue (~1.5 cm) surrounding the injection site in the upper jaw and the injection site, including the inferior alveolar nerve in the lower jaw.

U.S. Food and Drug Administration (FDA) Good Laboratory Practice Regulations (GLP) (21CFR Part 58), the Japanese Ministry of Health, Labor, and Welfare (MHLW) Good Laboratory Practice Standards Ordinance 21, and the Organization for Economic Cooperation and Development (OECD) Principals of Good Laboratory Practice [C(97)/186]. These studies were reviewed and received Institutional Animal Care and Use Committee (IACUC) approval.

Two GLP studies of systemic toxicity of and local tolerance to single and multiple submucosal injections of OraVerse were conducted. These studies were conducted in 35 and 15 beagle dogs, respectively, randomly assigned to one of the treatment groups comprising 7 and 3 subjects, respectively (Table 1A and B). The beagle dog was chosen as the test system because of its large mouth size and its established usefulness and acceptance as a model for toxicologic pharmacokinetic and pharmacologic studies. All animals in this study were naïve with respect to prior treatment. Animal records are maintained with the raw data.

The sample size for the second study was based on data from the first study. The low dose of phentolamine mesylate used in these studies (12 µg/kg) was equivalent to the highest dose studied in clinical trials (0.8 mg or 2 cartridges of OraVerse^{5,6}) based on a body surface area comparison between dogs and humans. The dogs received phentolamide HCl and HTAEDA at levels that were 2% and 3%, respectively, that of the phentolamine administered (according to the free base weights of the compounds). Each dog received treatment in 1 right maxillary quadrant and in 1 right mandibular quadrant (0.6 mL per 20 seconds). Injections of the lower jaw

were of the block type with a 27-gauge needle and was placed in the lingual tissue adjacent to the inferior alveolar nerve near its separation from the lingual nerve. Injections in the upper jaw were of the infiltration type given with a 30-gauge needle and placed adjacent to the first premolar. Three dogs per treatment group were sacrificed 24 hours (day 2) after treatment, 2 dogs at 72 hours (day 4) after treatment, and 2 dogs at 14 days (day 15) after treatment (Table 1A). In the multidose study, the animals were dosed on days 1, 8, and 15, and all were sacrificed at day 22 (Table 1B).

Systemic toxicity was evaluated through observation of clinical signs (daily), body weights (predose and weekly), food consumption (daily), hematology (predose and prior to necropsy), and blood chemistry (predose and prior to necropsy). During physical examinations, animals had the following parameters monitored: heart rate, body temperature, respiration, eyes, ears, skin and nails, oral cavity, abdominal palpation, lymph nodes, thoracic auscultation, gait, and disposition.

In both studies, submucosal tissue around the site of the injection was examined macroscopically (single-dose study: pretreatment and 10 minutes, 1, 3, and 24 hours post dosing and on days 3, 4, 8, and 15; multidose study: pretreatment and at 10 minutes, 1, 3, and 24 hours after each dose and on days 3, 4, 10, 11, 17, 18, and 22) and microscopically for morphologic changes.

For all animals, the inferior alveolar nerves were wet-trimmed to include a longitudinal and cross section in the same block. The blocks were processed, embedded in paraffin, and then sectioned to produce 2 slides. The first slide was taken when a complete section along the entire length of the nerve was ob-

Table 3. Organ Weight Tissues

Adrenals	Heart	Pituitary	Testes	Brain	Liver
Epididymides	Kidneys	Thyroid with parathyroid	Thymus	Spleen

Photomicrograph of normal (n) and degenerated nerve (d) adjacent in the gingival biopsy from an animal 24 hours after it received 27 µg/kg phentolamine mesylate in the right maxilla adjacent to the first premolar.

tained. The second section was taken after 50 four-micron step sections were cut. The alveolar branches of the superior alveolar nerves were wet-trimmed to include a single, longitudinal section, which was processed and embedded in paraffin. The first microtome section was taken when several nerve branches were apparent in the cut section, after which 3 additional 4-micron sections were collected after 25 four-micron step sections were cut between each section and saved. This produced a total of 4 sections with 100-micron intervals between each section collected. All slides then were stained with hematoxylin and eosin (H&E).

Tissues from the comprehensive tissue list (Table 2) from 6 dogs in each study were processed in the routine manner, stained with H&E, and evaluated microscopically. Because no pathology was observed in this representative sample, no additional tissue analysis was required. Results of all microscopic evaluations were recorded in a Provantis pathology data management system.

The organs listed in Table 3 were weighed from all animals. Paired organs were weighed together. Organ-to-body weight ratios were calculated from animals sacrificed at the scheduled necropsy.

Descriptive statistics (1-way analysis of variance and Dunnett's test) were computed for body weights, body weight changes, food consumption, hematology, and clinical chemistry parameters.

RESULTS

No deaths occurred in either study. No test article-related changes were noted in clinical signs, body

weight, food consumption, physical examination parameters, or ophthalmologic examination findings. Although clinical pathology analyses revealed significant changes compared with the control group in the multidose study, these changes were all present at the prestudy evaluation and were not related to the test article administration. Statistically significant changes in hematology parameters, which were present at the $P \leq .05$ level, consisted of prestudy increased mean hemoglobin (Groups 3 [16.6 g/dL], 4 [16.2 g/dL], and 5 [15.8 g/dL]) and increased mean hematocrit (Groups 3 [51.0%] and 4 [50.2%]) from Group 1 (hemoglobin: 14.9 g/dL; hematocrit: 46.2%). Mean values were within the normal range (hemoglobin: 10 to 21.6 g/dL; hematocrit: 32.9 to 61.2%) for this laboratory.

At the necropsy evaluation, no statistically significant changes in hematology, serum chemistry, coagulation, organ weights, and organ-to-body weight ratios, or microscopic changes, were seen in tissues listed in Table 2. No clinically relevant changes were noted in urinalysis parameters evaluated 0 to 6 hours after the single dose in the first study or after the third dose in the multidose study.

Nerve fiber degeneration was microscopically evident in several branches of the superior alveolar nerve near the injection site in 1 Group 2 dog that received phentolamine mesylate (12 µg/kg), HTAEDA (0.27 µg/kg), and phentolamide HCl (0.2 µg/kg) from the single-dose study and was euthanized approximately 24 hours after test article administration (Figure). Several branches of the superior alveolar nerve in this dog were present in the sections available for examination. The severity of the degeneration was moderate and was confined to segments of only a minority of the branches. Nerve fiber changes were characterized by axonal degeneration and fragmentation with myelin swelling. No other degenerative or inflammatory changes were evident in tissues surrounding the affected nerves. The inferior alveolar nerve in this dog was unaffected.

Nerve fiber degeneration of moderate severity and chronic perineural inflammation were also present in the inferior alveolar nerve near the injection site in 1 control dog in the multidose study. Chronic inflammation also was observed in that animal in the perineural tissue adjacent to the nerve. The inflammation involved the perineurium at 1 site only (data not shown). In addition to the inflammation described above for the control dog, chronic inflammation of minimal or mild severity was present in perineural tissues at the mandibular injection site adjacent to the inferior alveolar nerve in 1 dog each from Groups 3 and 5 and in 2 dogs from Group 4. Fibrinoid vasculitis was associated with inflammation in 1 Group 3 dog.

Lymphohistiocytic or mixed inflammation was observed in subcutaneous tissues at the maxillary injection site in 1 dog from each of the 5 treatment groups. The inflammation was always of minimal severity. All of these animals were from the multidose study.

DISCUSSION

The nature of the changes to the nerves and perineural tissue and their presence in 1 control dog indicate that lesions at both injection sites apparently were associated with needle penetration during injections and were not a direct effect of the test materials. In addition, (1) no degenerative changes were noted in dogs from the highest-dose group in which all test articles were given at 10 times the dosage given to the dogs in Group 2; (2) no degeneration was seen in the inferior alveolar nerves at any dose level; (3) only a few of numerous nerve branches at the site were involved; (4) no other inflammatory or degenerative lesions were observed in tissues surrounding the affected nerves; (5) only a single site was involved; and (6) degeneration occurred in only 1 dog.

No other treatment-associated microscopic changes were noted in inferior or superior alveolar nerves from dogs in all groups. These data indicate that single and repeated intraoral administration of phentolamine mesylate, HTAEDA, and phentolamide HCl in beagle dogs is well tolerated.

Phentolamine mesylate has been studied in 6 independent genetic toxicity studies and was found to be negative for genotoxicity and chromosomal clastogenesis. HTAEDA and phentolamide HCl were tested similarly and were found to be neither genotoxic nor clastogenic (Novalar Pharmaceuticals, Inc, unpublished

data). These data, combined with strong clinical safety data obtained in 1 phase 2 (adults),⁶ 2 phase 3 (adolescents and adults),⁵ and 1 phase 2 study in pediatric patients,⁴ suggest that minimal risk is associated with intraoral submucosal injections of OraVerse.

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