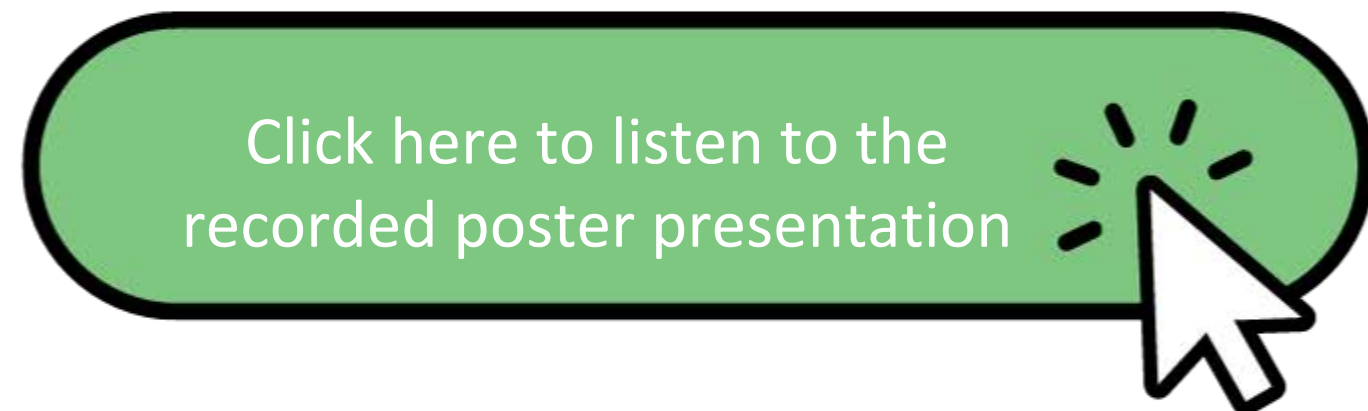


Creation of a Swine Model of Oral Angioedema

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ABSTRACT

Angioedema is a sudden swelling often caused by allergic reactions, commonly affecting the face, particularly the lips and tongue. Angioedema in this area may be life-threatening, especially if the throat is affected and breathing obstructed. While there are miniature swine (MS) models to induce swelling using histamine injections, there is no large animal model for angioedema in the oral cavity. In the current study, 6 male Yucatan MS were injected in the mucosa of the inner lip (left and right) and intramuscularly into the tongue with 20 µL injections at different dose concentrations of histamine dihydrochloride in a dose-escalating design at 0.0005, 0.0025, 0.01, 0.02, 0.1, and 0.2 mg/injection site. Animals were anesthetized with isoflurane to ensure the accuracy of the dose administration and measurements. Digital calipers were used to measure tissue swelling and Draize scores were performed to monitor erythema and edema around each injection site. The animal dosed at 0.2 mg/injection site, the highest concentration (10 mg/mL), displayed the desired reaction and an additional 4 male animals were dosed at this level to ensure reproducibility of results. For the 5 animals dosed at 0.2 mg/injection site, the majority of swelling in both the lips and tongue occurred within the first 30 minutes. Lip thickness continued to mildly increase from 30 to 90 minutes, whereas swelling in the tongue decreased from 30 to 90 minutes, though not back to baseline. Draize scores showed increased erythema and edema starting at 10 minutes. Animals were given diphenhydramine during anesthetic recovery to reduce any remaining swelling and all animals recovered uneventfully. The data demonstrate that histamine dihydrochloride serves as a suitable agent for inducing angioedema in the tongue and lips of MS and can serve as a valuable large animal model for assessing treatments of angioedema and investigating the impact of angioedema on drug absorption when administered sublingually and/or buccally.

METHOD

Male Yucatan miniature swine weighing 46.1 to 75.9 kg at an age of 3.7 to 4.3 years of age were fasted for a period of at least 8 hours prior to dosing. Anesthesia was induced with a combination of Telazol (26 mg/kg, IM) and Xylazine (0.4-1.2 mg/kg, IM), and animals were intubated for maintenance of anesthesia with isoflurane (up to 5% in 100% oxygen) to facilitate administration of histamine dihydrochloride and subsequent observations/measurements. Injections into the mucosal surface of the inner lip (left and right side) were administered at a target depth of 3 mm. Intramuscular injections into the tongue were administered at a target depth of 3 mm. The needle was marked with a sterile marker to the desired depth to facilitate appropriate injection depth.

Thickness of the lip and tongue were measured with digital calipers prior to dose and at 10, 20, 30-, 40-, 60-, and 90 minutes post-dose. The inner lip and tongue were observed for irritation using the Modified Draize scoring system prior to dose and at 10-, 30-, 60-, and 90 minutes post-dose.

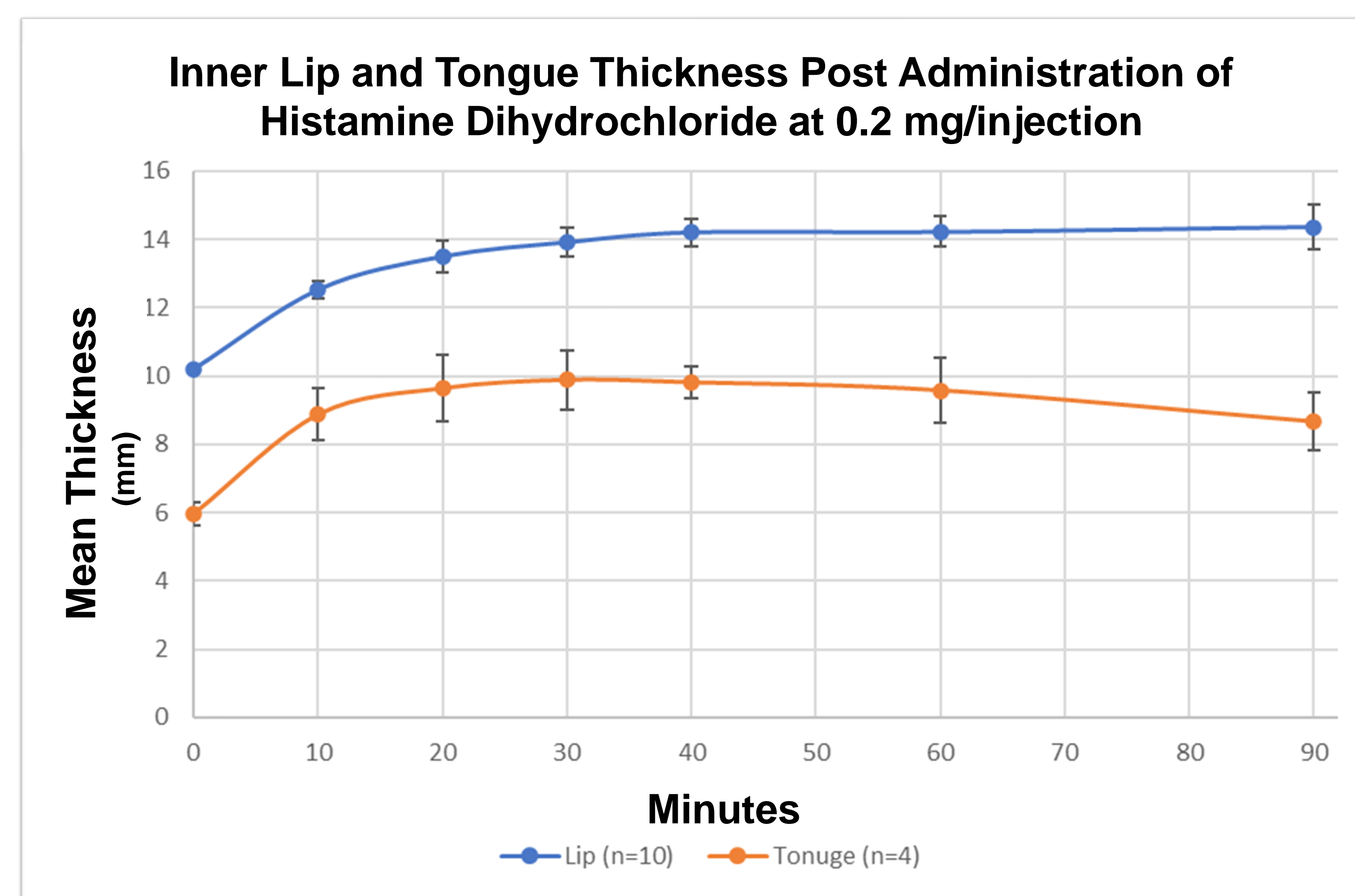


Figure 1. 5 animals were given histamine dihydrochloride injections at 0.2 mg/injection. The left and right side of the lower lip was measured resulting in N=10 measurements for the lip. One animal did not have a reaction at the tongue. This is likely due to a mis-injection and resulted in N=4 measurements being presented.

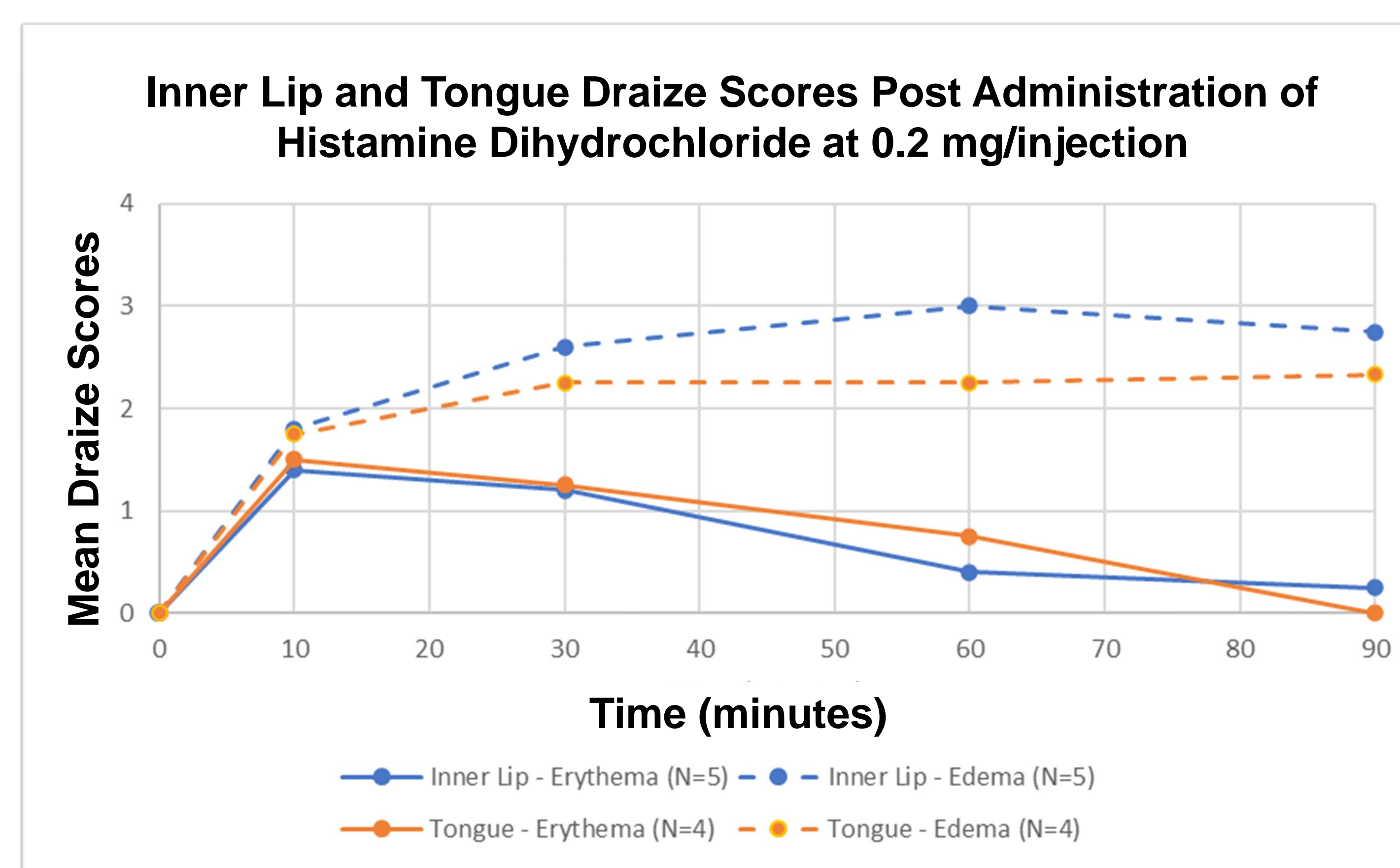


Figure 2. One animal did not have a reaction at the tongue. This is likely due to a mis-injection.

Table 1. Modified Draize Scoring System

Category	Score	Description
Erythema	0	None
	1	Slight
	2	Well-defined
	3	Moderate to severe
	4	Severe or slight eschar formation (injuries in depth)
Edema	0	None
	1	Very slight
	2	Slight (well-defined edges)
	3	Moderate (raised > 1 mm)
	4	Severe (raised > 1 mm and extending beyond the area of exposure)

RESULTS

- Administration of histamine dihydrochloride at dose levels of 0.0005, 0.0025, 0.01, .02, and 0.1 mg/injection site did not display the desired reaction
- Administration of histamine dihydrochloride at a dose level of 0.2 mg/injection site did display the desired reaction
- A total of 5 animals were dosed with histamine dihydrochloride at a concentration of 0.2 mg/injection site to ensure reproducibility
- The majority of swelling in both the lips and tongue occurred within the first 30 minutes
- Lip thickness continued to mildly increase from 30 to 90 minutes, whereas swelling in the tongue decreased from 30 to 90 minutes, though not back to baseline
- Draize scores showed increased erythema and edema of the inner lip and tongue starting at 10 minutes
- Animals were given diphenhydramine during anesthetic recovery to reduce any remaining swelling and all animals fully recovered

CONCLUSION

The desired effect of angioedema was achieved following injections of 0.2 mg histamine dihydrochloride into 3 locations (the mucosal surface of the right inner lip, the mucosal surface of the left inner lip, and the tongue) at a target depth of 3 mm. The optimal dose of 0.2 mg histamine dihydrochloride was tested in 5 animals which displayed the desired level of angioedema based on physical measurements and Draize scoring. The creation of this animal model makes it possible to conduct efficacy studies that will more closely mimic allergic reactions in the clinic and to assess treatments administered in the oral cavity and the potential impact of absorption when localized swelling of the oral mucosa occurs.