The impact of anaphylaxis on the absorption of intranasal epinephrine in anaesthetized non-naive beagle dogs

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Background: Epinephrine delivery via an intranasal spray (*neffy*) is being evaluated as an additional option to treat severe allergic reaction and may provide clinical benefit by reducing the time to dosing in community settings by avoiding needles. Given that hypotension is a hallmark symptom of severe allergic reactions, a preclinical study was conducted to evaluate the impact of this factor on epinephrine absorption via *neffy*.

Objective: The objective of this study was to evaluate the absorption of epinephrine via *neffy* in a dog model of anaphylaxis with severe hypotension.

Methods: Epinephrine absorption via *neffy* was evaluated in anesthetized beagle dogs under both normal conditions and hypotension associated with anaphylaxis. A total of 14 dogs (10 males and 4 females) were dosed with *neffy*, 1.0 mg, under normal conditions, followed by *neffy*, 1.0 mg, under conditions of anaphylaxis.

Results: The mean maximum concentration of epinephrine was higher during anaphylaxis than under normal conditions (2,670 ± 2,150 pg/mL and 1,330 ± 739 pg/mL [$P < .05$]). Relative to normal conditions, anaphylaxis resulted in higher overall epinephrine exposure (area under the curve from 0 to 45 minutes = 54,400 ± 18,100 min × pg/mL and 34,300 ± 21,500 minutes × pg/mL [$P < .05$]), which is likely due to the increase in vascular permeability commonly observed during severe allergic reactions.

Conclusion: Taken together with real-world evidence from nasal naloxone treatment for opioid overdose demonstrating that the reduced blood flow or hypotension associated with overdose does not appear to suppress naloxone's efficacy, the current findings demonstrate that epinephrine is well absorbed following *neffy* delivery during the hypotension associated with severe anaphylaxis reactions. (J Allergy Clin Immunol Global 2023;2:100165.)

Key words: *Epinephrine, nasal congestion, intranasal, severe allergy, anaphylaxis, allergy, hypotension*

Abbreviation used
GLP: Good Laboratory Practice

INTRODUCTION

Epinephrine is the first-line treatment for severe allergic reactions and anaphylaxis.[1] Epinephrine autoinjectors are the most frequently used products for out-of-hospital treatment; however, patients and caregivers are reluctant to use injectable products. Up to 83% of patients and caregivers have reported failing to administer or delaying use of epinephrine autoinjectors, even when they know that they are having a severe allergic reaction.[2-5] Treatment delays may increase the risk of death by airway obstruction or vascular collapse.

neffy is an intranasal epinephrine spray being developed as an alternative to intramuscular injection. *neffy* is expected to have significant clinical benefit by reducing treatment delays. A series of clinical trials have demonstrated that *neffy* is safe and well tolerated, with a pharmacokinetic profile within the range of the profile of currently approved products. This preclinical study was conducted to assess the impact of severe anaphylaxis, including hypotension, on the absorption of epinephrine via *neffy*. Because conducting clinical trials in patients with anaphylaxis is unethical, this Good Laboratory Practice (GLP) study was conducted using a dog anaphylaxis model.

The study was performed at Charles River Laboratories in accordance with the Organization for Economic Co-operation and Development Principles of GLP and as accepted by regulatory authorities, including the US Food and Drug Administration. All experimental procedures were performed in accordance with the Institutional Animal Care and Use Committee and the Canadian Council on Animal Care guidelines for the use of experimental animals. All protocols included humane euthanasia criteria and were reviewed and approved by the Institutional Animal Care and Use Committee. Animals were continuously monitored by technical and veterinary staff to ensure animal welfare and promptness of care when applicable.

The objective of this study was to evaluate the pharmacokinetics of *neffy* in anesthetized beagle dogs under both normal and Tween 80–induced anaphylaxis conditions.[6] A total of 14 dogs (10 males and 4 females) were dosed with *neffy,* 1.0 mg, under normal conditions, followed by *neffy,* 1.0, mg under conditions of anaphylaxis. Treatments were separated by a minimum of 7 days. Either a 0.9% saline solution in a concentration of 1.2 mL/kg (to model normal conditions) or 0.25% Tween 80 (to model anaphylaxis) was administered intravenously 7 minutes before administration of *neffy*. The dog model has been well characterized for the study of intranasal drug administration.[7]

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Anaphylaxis was scored as follows: 0, no signs of anaphylaxis; 1, minimal to slight skin erythema (redness) or minimal skin edema (swelling); 2, moderate skin erythema and/or skin edema; 3, severe erythema and/or edema without signs of hypotension; and 4, signs of hypotension reported under anaphylaxis.

Histamine levels were used to confirm anaphylaxis. During anaphylaxis, blood samples were collected at 60 minutes and 3 minutes before *neffy* administration and at 5, 10, 15, 30, 45, and 60 minutes after *neffy* administration; the time point –3 minutes was 4 minutes after Tween 80 administration. Samples were analyzed using a validated ELISA method.

For both sessions, blood samples for epinephrine were collected at 60 minutes and 3 minutes before administration of *neffy*, which was 4 minutes after Tween 80 for anaphylaxis, and at 2.5, 5, 7.5, 10, 15, 20, 30, and 45 minutes after *neffy*. Epinephrine plasma concentrations were determined by Worldwide Clinical Trials, Early Phase Services, and Bioanalytical Sciences by using a liquid chromatography coupled to tandem mass spectrometry (LC MS/MS) method (ATM-2663) with an analytic range of 100 to 20,000 pg/mL. Observed and baseline-adjusted epinephrine plasma concentration time data for individual animals were analyzed by using noncompartmental methods in Phoenix WinNonlin (version 8.1, Certara, LP) in conjunction with the Pharsight Knowledgebase Server (PKSO; version 4.0.4, Certara, LP). During pharmacokinetic analysis, plasma concentrations below the lower limit of quantification (<100 pg/mL) were treated as one-half the lower limit of quantitation (50.0 pg/mL). One-way ANOVA was used to compare groups.

RESULTS AND DISCUSSION

Two animals were excluded from the analysis in the anaphylaxis session for the following reasons: the first was excluded because at 2.5 minutes following administration of *neffy*, it had an epinephrine concentration more than 10-fold higher than the mean concentration in the other subjects (37,100 pg/mL vs 1,670 pg/mL), and the second was excluded because at baseline (3 minutes before *neffy* administration), it had an epinephrine concentration of 4,050 pg/mL, which was 6.5 times higher than the mean concentration in the other subjects (616.3 pg/mL), which was possibly a response to severe hypotension (37/25 mm Hg at 0 minutes).

All of the dogs showed signs of anaphylaxis following administration of Tween 80. On the basis of the maximum score within 30 minutes of Tween 80 administration, the median anaphylaxis score was 2 (moderate skin erythema and/or skin edema), with the individual scores ranging from 1 to 4.

Successful induction of anaphylaxis was confirmed by increases in histamine levels after Tween 80 administration. Increased histamine levels were noted in all of the animals, with the changes ranging from 57.9 to 230.5 times baseline.

The baseline mean (± SD) systolic/diastolic blood pressure was 113 (± 47)/62 (± 27) mm Hg before anesthesia induction; it decreased to 94 ± 16/55 ± 13 mm Hg following general anesthesia.

For anaphylaxis session, the mean (± SD) systolic/diastolic blood pressure was 137 (± 50.4)/78 (± 30) mm Hg before anesthesia induction (and Tween 80 administration); it decreased to 61 (± 10)/39 (± 7) mm Hg following anesthesia induction and Tween 80 administration.

The greater decrease seen during anaphylaxis represents the combined effect of anesthesia and anaphylaxis.

At 60 minutes before *neffy* administration (and before induction of anesthesia under normal conditions and conditions of anesthesia with Tween 80 administration for anaphylaxis), the mean baseline epinephrine concentrations of the normal and anaphylaxis conditions were similar (59.1 ± 23.3 pg/mL and 61.2 ± 26.0 pg/mL, respectively).

At –3 minutes, the epinephrine concentration under normal conditions was 50.0 ± 0 pg/mL. Anaphylaxis induction resulted in a marked increase in epinephrine level (from 61.2 ± 26.0 pg/mL to 566.8 ± 843.4 pg/mL) (Table I). This increase occurred before *neffy* administration and was most likely due to stress, including a response to an anaphylaxis-related decrease in blood pressure.

Following *neffy* administration, the baseline adjusted mean epinephrine maximum concentration was higher during anaphylaxis than under normal conditions (2,670 ± 2,150 pg/mL and 1,330 ± 739 pg/mL [$P < .05$]). Relative to normal conditions, anaphylaxis resulted in higher overall epinephrine exposure (area under the curve from 0 to 45 minutes = 54,400 ± 18,100 min × pg/mL and 34,300 ± 21,500 min × pg/mL [$P < .05$]) (Table II). The higher epinephrine concentrations during anaphylaxis were most pronounced at early time points (Fig 1), suggesting that anaphylaxis increases the rate of epinephrine absorption immediately after dosing. Similar trends were noted for the non–baseline-adjusted epinephrine data.

In this GLP preclinical study, epinephrine absorption via *neffy* was increased during anaphylaxis, the symptoms of the increase included erythema, edema, and hypotension. In a previous non-GLP study, anaphylaxis was induced in 7 animals (via Tween 80), and the mean observed maximum concentration after anaphylaxis (without *neffy* administration) was 601 ± 442 pg/mL (data not shown), which is similar to the result in the current study (566.8 ± 843.4 pg/mL at –3 minutes). The comparable increase in epinephrine levels following anaphylaxis in the absence of *neffy* in both the non-GLP and GLP studies suggests that the more marked increase in the current study was attributable to the administration of epinephrine via *neffy* and not to the stress of anaphylaxis.

Clinical studies in healthy volunteers have demonstrated that epinephrine absorption via *neffy* is comparable to that from approved injection products.[8,9] Although currently there are no published studies examining the effect of severe hypotension on epinephrine absorption following intranasal administration, the data regarding the impact of hypotension on nasal absorption following administration of naloxone nasal spray during opioid overdose suggest that there is no meaningful relationship between hypotension and intranasal absorption. During acute opioid overdose, patients are typically experiencing respiratory depression or cardiac failure and moderate-to-severe hypotension.[10-12] Clinical studies of naloxone treatment during overdose have demonstrated that intranasal naloxone is equal to or more effective than injection.[13-17] In a randomized study comparing intranasal and intravenous administration of naloxone in patients with opioid overdose, in which baseline systolic blood pressure was less than 100 mm Hg and arterial O_2 saturation was approximately 70%, intranasal naloxone demonstrated equivalent or better outcomes.[18] Given that opioid-induced hypotension may be mediated by histamine release,[19-23] the efficacy of intranasal naloxone for out-of-

TABLE I. Epinephrine concentrations before and after anaphylaxis induction

Session	n	Time relative to *neffy* administration (pg/mL), mean epinephrine concentration (SD)	
		–60 min (before introduction of anesthesia and anaphylaxis)	–3 min (after saline administration or after anaphylaxis induction)
neffy, 1.0 mg, during normal conditions	14	59.1 (23.3)	50.0 (0.0)
neffy, 1.0 mg, during anaphylaxis	12	61.2 (26.0)	566.8 (843.4)

TABLE II. Baseline corrected epinephrine parameters following *neffy,* 1.0 mg

Session	n	^{C}max (pg/mL), ^{mean} (SD)*	^{T}max (min), m^{edian} (range)	^{AUC}0-45 (min × pg/mL), ^{mean} (SD)*
neffy, 1.0 mg, during normal conditions	14	1,330 (739)	7.5 (2.5-45)	34,300 (21,500)
neffy, 1.0 mg, during anaphylaxis	12	2,670 (2,150)	10 (2.5-45)	54,400 (18,100)†

$AUC_{0\text{-}45}$, Area under the curve from 0 to 45 minutes; C_{max}, maximum concentration; T_{max}, time to maximum concentration.
*$P < .05$.
†n = 9, as baseline-corrected epinephrine concentration was 0 in 3 animals and AUC0-45 was not determined.

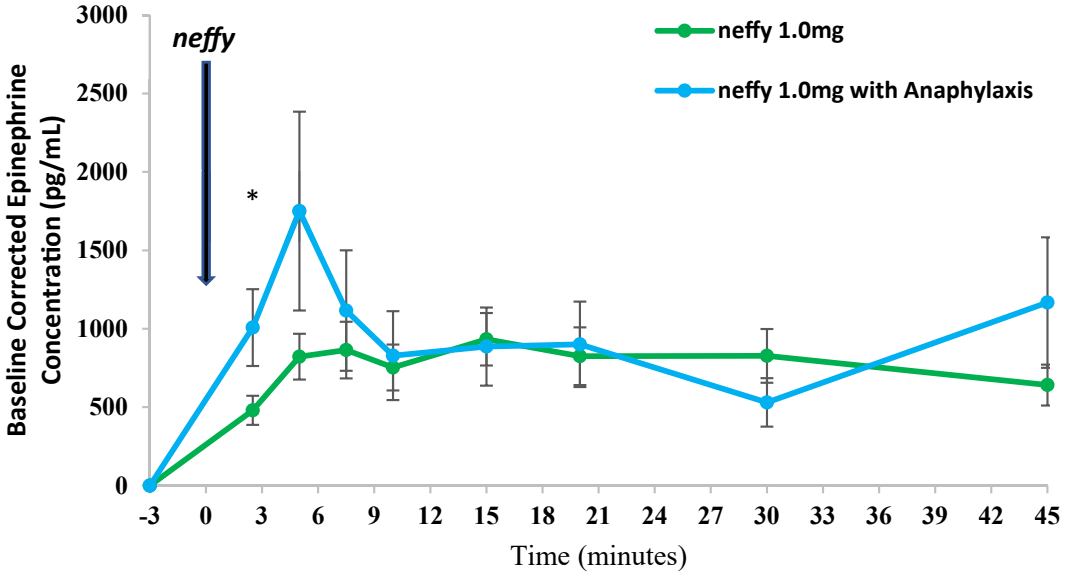

FIG 1. Baseline corrected epinephrine concentrations are plotted over time (means ± SEMs). The higher epinephrine concentrations during anaphylaxis relative to those under normal conditions were most pronounced at early time points. Arrow indicates administration of *neffy* at 0 minutes. *$P < .05$.

hospital use suggests that intranasal epinephrine will be well absorbed during histamine-mediated hypotension.

In this study, the absorption of epinephrine during anaphylaxis and associated hypotension was not only not affected but was in fact increased. This increased absorption may be attributable to the release of vasoactive mediators such as histamine, which increase vascular permeability.[1,24] Increased epinephrine absorption has also been reported under histamine-induced nasal congestion in dogs.[25] These data suggest that intranasal administration of epinephrine may be enhanced during the increased permeability associated with anaphylaxis.

Epinephrine absorption following *neffy* does not appear to be affected by hypotension caused by anaphylaxis and may be enhanced by an increase in vascular permeability. This is consistent with real-world evidence from nasal naloxone, demonstrating that reduced blood flow/hypotension associated with opioid overdose does not appear to suppress naloxone's efficacy. Additionally, the current data demonstrate that *neffy* absorption appears to be enhanced by anaphylaxis, likely thanks to the increased vascular permeability commonly observed during anaphylaxis. These findings support *neffy*'s potential efficacy during hypotension associated with severe allergic reactions.

DISCLOSURE STATEMENT

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Disclosure of potential conflict of interest: S. Tanimoto and R. Lowenthal are employees of ARS Pharmaceuticals. The rest of the authors declare that they have no relevant conflicts of interest.

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Clinical implications: Effective treatment of anaphylaxis is dependent on the rapid absorption of epinephrine. The results of this study demonstrate that epinephrine is well absorbed in the presence of hypotension associated with anaphylaxis.

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