Pharmacokinetics/pharmacodynamics of epinephrine after single and repeat administration of *neffy*, EpiPen, and manual intramuscular injection

Thomas B. Casale, MD,[a] Anne K. Ellis, MD,[b] Anna Nowak-Wegrzyn, MD, PhD,[c] Michael Kaliner, MD,[d] Richard Lowenthal, MS, MBA,[e] and Sarina Tanimoto, MD, PhD[e] *Tampa, Fla; Kingston, Ontario, Canada; New York, NY; Wheaton, Md; and San Diego, Calif*

Background: Epinephrine is the first-line treatment for severe allergic reactions, and rapid treatment is associated with lower rates of hospitalization and death. Current treatment options (epinephrine auto-injectors and manual intramuscular injection) are considered cumbersome, and most patients/caregivers fail to use them, even during severe reactions. An intranasal epinephrine delivery device, *neffy*, has been designed to provide an additional option for patients/caregivers.
Objective: We sought to assess the comparative pharmacokinetics and pharmacodynamics of *neffy* 2.0 mg, EpiPen 0.3 mg, and manual intramuscular injection 0.3 mg.
Methods: This was a phase 1, randomized, 6-treatment, 6-period, 2-part crossover study in 59 healthy subjects. Pharmacokinetic and pharmacodynamic parameters following single and repeat doses of epinephrine were assessed before dosing and at various postdose intervals.
Results: The pharmacokinetic profile of *neffy* was bracketed by approved injection products, with a mean peak plasma level of 481 pg/mL, which fell between EpiPen (753 pg/mL) and epinephrine manual intramuscular injection (339 pg/mL). When dosed both once and twice, *neffy* resulted in more pronounced increases in pharmacodynamic parameters relative to EpiPen or manual injection.
Conclusions: *neffy*'s pharmacokinetic profile was bracketed by approved injection products, with pharmacodynamic responses that were comparable to or better than approved injection products. *neffy* is expected to be a safe and effective option, particularly for patients/caregivers who are reluctant to carry and use injection devices. (J Allergy Clin Immunol 2023;■■■:■■■-■■■.)

Key words: *Anaphylaxis, drug allergy, epinephrine, intranasal, intranasal epinephrine*

Epinephrine is the first-line treatment for severe allergic reactions, including anaphylaxis.[1-3] Several epinephrine injection products are approved for use; however, notable differences in their pharmacokinetic (PK) and pharmacodynamic (PD) profiles have been reported.[4-7] Despite this, all approved epinephrine products are considered efficacious for the treatment of severe allergic reactions/anaphylaxis.

Although epinephrine auto-injectors (EAIs) are safe and effective for the treatment of anaphylaxis, they are frequently considered inconvenient and cumbersome. Up to 83% of patients/caregivers fail to administer or delay using EAIs even when they know a severe allergic reaction is occurring.[8-10] Barriers to using EAIs include high costs, unwillingness to carry the device, failure to recognize a severe allergic reaction, fear of needles, and lack of proper training.[11-13] There remains an unmet need to provide an additional needle-free epinephrine delivery option for the first-line treatment of allergic reactions/anaphylaxis.

The *neffy* (ARS Pharmaceuticals, Inc, San Diego, Calif) device is an intranasal (IN) needle-free epinephrine nasal spray that is being developed for the emergency treatment of (type I) allergic reactions, including anaphylaxis. A needle-free epinephrine delivery device is expected to have clinical benefits by reducing treatment apprehension and delay, reducing accidental injections, and making it easier to carry the product.[14] Because it is not possible to conduct prospective clinical trials in patients experiencing anaphylaxis, the development strategy is to 1) demonstrate that the PK profile of *neffy* is within the range of currently approved injection products (manual intramuscular [IM] injection and Food and Drug Administration-approved EAIs) and 2) use PD data as a surrogate for efficacy. This study evaluated the PK and PD of *neffy* 2.0 mg compared with EpiPen (Mylan Specialty, Morgantown, WV) 0.3 mg (EpiPen) and Epinephrine 0.3 mg via manual IM injection (Epinephrine IM) with needle and syringe.

METHODS

Study design and participants

This study was approved by the Alpha Independent Review Board and conducted in accordance with Good Clinical Practice and the Declaration of Helsinki. All subjects provided written informed consent before screening.

From [a]Morsani College of Medicine, University of South Florida, Tampa; [b]Kingston General Health Research Institute, Kingston; [c]NYU Grossman School of Medicine, New York; [d]Institute for Asthma & Allergy, Wheaton; and [e]ARS Pharmaceuticals, San Diego.

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Corresponding author: Sarina Tanimoto, MD, PhD, ARS Pharmaceuticals, Inc, 11682 El Camino Real, Suite 120, San Diego, CA 92130. E-mail: sarinat@ars-pharma.com.

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Abbreviations used

AUC: Area under the curve
BP: Blood pressure
C_{max}: Maximum plasma concentration
DBP: Diastolic blood pressure
EAI: Epinephrine auto-injector
E_{max}: Maximum effect
EpiPen: EpiPen 0.3 mg
Epinephrine IM: Epinephrine 0.3 mg via manual intramuscular injection
HR: Heart rate
IM: Intramuscular
IN: Intranasal
L/R: Left/right
PD: Pharmacodynamics
PK: Pharmacokinetics
R/R: Right/right
SBP: Systolic blood pressure
T_{max}: Time to C_{max}
T_{Emax}: Time to maximum effect

This was a phase 1, 6-treatment, 6-period, crossover study consisting of screening and baseline periods and an open-label randomized treatment period. The study was conducted in 2 parts, with single doses of epinephrine administered in part 1 and repeated doses administered 10 minutes apart in part 2. In part 1, each treatment was separated by 24 hours. In part 2, each treatment was separated by at least 6 days. Parts 1 and 2 were separated by 12 days. In part 1, subjects were randomly assigned to receive a single dose of *neffy* 2.0 mg/100 μL, a single dose of EpiPen in the anterolateral thigh, and a single dose of Epinephrine IM with a 22-gauge, 1-inch needle in the anterolateral thigh. In part 2, subjects were randomly assigned to receive 2 doses of *neffy* 2.0 mg in the right naris (R/R); 2 doses of *neffy* 2.0 mg, one in the left naris and one in the right naris (L/R); and two doses of EpiPen, one in the left anterolateral thigh and one in the right thigh (L/R).

PK and PD assessments

Blood samples were collected before dosing and at 2, 4, 6, 8, 10, 12.5, 15, 20, 30, 45, 60, 90, 120, 150, 180, 240, and 360 minutes after dosing. Plasma epinephrine concentrations were determined using a validated liquid chromatography-mass spectrometry/mass spectrometry method with a range of quantitation of 20.0 to 4000 pg/mL. Epinephrine concentration and PK parameters were calculated without subtracting predose epinephrine levels, as absolute plasma levels are considered more clinically relevant. PK parameters included maximum plasma concentration (C_{max}), time to C_{max} (T_{max}), and area under the curve from time-zero to the time of the last quantifiable concentration (AUC_{last}).

PD parameters, including systolic blood pressure (SBP), diastolic blood pressure (DBP), and heart rate (HR), were measured using an automated blood pressure (BP) measuring device. BP and HR were measured at baseline; before dosing; and at 1, 5, 10, 15, 20, 25, 30, 45, 60, 90, and 120 minutes after dosing. PD data were expressed as change from baseline. Maximum effect (E_{max}), time to maximum effect (T_{Emax}), and the relationship between C_{max} and E_{max} were analyzed to assess PD differences among products.

Data were analyzed using noncompartmental methods in Phoenix WinNonlin (Version 8.1, Certara, Princeton, NJ) in conjunction with the internet-accessible implementation of Pharsight Knowledgebase Server (PKSO; Version 4.0.4, Certara).

Safety assessments

Safety assessments included adverse events, vital signs, nasal irritation and pain, and physical examinations.

Statistical analysis

PK and PD parameters were summarized with descriptive statistics, including arithmetic mean, SD, median, minimum, maximum, and coefficient of variation. For PK analyses, the natural logarithmic-transformed PK parameters C_{max} and AUC_{last} were analyzed across treatments using a linear mixed effects model with sequence, subject within sequence, period, and treatment as fixed effects and subject as the random effect. CIs (90%) were constructed for the geometric mean ratios. Statistical analysis was conducted using linear mixed effects. The nontransformed PD parameters E_{max} and AUC_{last} were analyzed across treatments using a linear mixed effects model as described for the PK analysis but without data transformation. The least squares means, differences in least squares mean between test and reference, 90% CIs, and *P* values were calculated. *P* values presented in this article were not adjusted for multiple testing. A study population of 30 subjects was considered adequate based on power calculations (type 1 error rate of 5%). The number of subjects was considered adequate for safety evaluations.

RESULTS

Study participants

The study was planned to enroll 42 subjects. However, due to a PK processing error, PK samples from cohort 1 (n = 14) in part 1 were unusable, and an additional cohort (n = 14) was added to replace them for a total of 42 subjects in part 1 that included 5 returning subjects from cohort 1. Before part 2 began, 3 subjects withdrew and were replaced before dosing. Before the final (sixth) dosing, 6 additional subjects withdrew, 3 for *neffy* 2.0 mg (L/R) and 3 for (R/R), resulting in 39 subjects receiving *neffy* 2.0 mg (L/R) and (R/R) and 42 subjects receiving EpiPen for PK/PD analysis. A total of 59 subjects (42 + 14 + 3) were included in the analysis, of which 54 were unique.

Subjects ranged in age from 21 to 54 years; 38 subjects (70.4%) were male, and 16 (29.6%) were female. Regarding race, 30 subjects (55.6%) were White, 17 subjects (31.5%) were Black, 4 subjects (7.4%) were Asian, and 3 subjects (5.6%) self-reported as "other." Subjects had a mean (SD) height of 172.0 (10.19) cm, a mean (SD) weight of 82.7 (12.52) kg, and a mean (SD) body mass index of 28.0 (3.24) kg/m^2.

PK results

Following a single dose, mean epinephrine concentrations were highest for EpiPen until approximately 20 minutes after dosing (Fig 1). From 30 to 360 minutes after dosing, greater mean epinephrine concentrations were observed with *neffy* compared with EpiPen and Epinephrine IM. Following repeated doses,

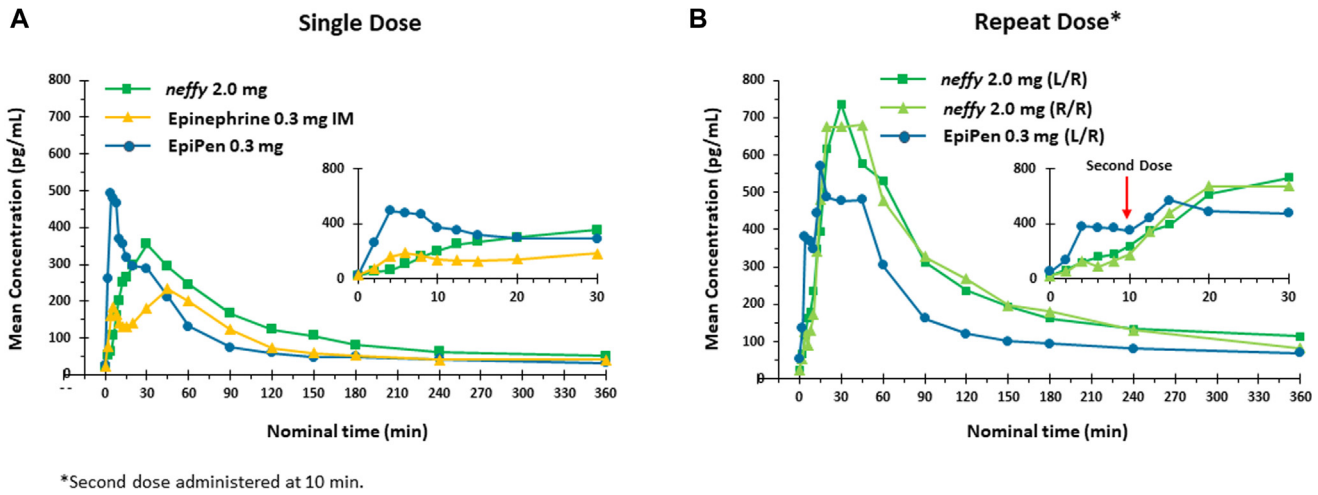

FIG 1. Mean epinephrine concentration-time profiles. **(A)** Single dose. **(B)** Repeat dose.

TABLE I. Summary statistics of epinephrine PK parameters

Treatment	No. of subjects	C_{max} (pg/mL), mean (CV%)	T_{max} (min), median (range)	AUC_{last} (min × pg/mL), mean (CV%)
Single dose				
neffy 2.0 mg	42	481 (76.0)	30.0 (6.00-150)	43,500 (69.4)
EpiPen	42	753 (65.6)	7.50 (2.00-45.0)	31,300 (35.0)
Epinephrine IM	42	339 (74.1)	45.0 (4.00-90.0)	29,300 (41.7)
Repeat dose				
neffy 2.0 mg (L/R)	39	1,000 (93.1)	30.0 (6.00-150)	86,000 (77.0)
neffy 2.0 mg (R/R)	39	992 (75.3)	30.0 (4.00-150)	86,000 (60.5)
EpiPen (L/R)	42	840 (60.6)	15.0 (0.00-360)	56,900 (52.1)

CV%, Coefficient of variation.

greater mean epinephrine concentrations were observed with both *neffy* treatments (R/R and L/R) compared with EpiPen.

Following a single dose, mean C_{max} values were highest with EpiPen, followed by *neffy* and Epinephrine IM, with a statistically significant difference between EpiPen versus *neffy* ($P = .0001$), but not *neffy* versus Epinephrine IM (Table I). Median T_{max} was fastest for EpiPen, followed by *neffy* and Epinephrine IM, with a statistically significant difference between EpiPen versus *neffy* ($P < .0001$), but not *neffy* versus Epinephrine IM. The greatest total exposure was observed for *neffy*, followed by EpiPen and Epinephrine IM, with no statistically significant difference between EpiPen versus *neffy* and with a statistically significant difference between *neffy* versus Epinephrine IM ($P = .0187$).

Following repeated doses, C_{max} values were highest with *neffy* (L/R) and (R/R), followed by EpiPen. There was no statistically significant difference between *neffy* (L/R) versus *neffy* (R/R), *neffy* (R/R) versus EpiPen (L/R), and *neffy* (L/R) versus EpiPen (L/R). Median T_{max} was fastest for EpiPen versus *neffy* (L/R) and *neffy* (R/R), but there were no statistically significant differences. Mean total exposure was lower following EpiPen compared with *neffy* (R/R) and *neffy* (L/R), with a statistically significant difference between *neffy* (R/R) versus EpiPen (L/R) and *neffy* (L/R) versus EpiPen (L/R), but not for *neffy* (L/R) versus (R/R).

PD results

All treatments resulted in an increase from baseline SBP, with the greatest increase observed following *neffy* (Fig 2 and Table II). EpiPen was associated with a less pronounced and more abrupt increase relative to *neffy*; a nominal change in SBP was observed following Epinephrine IM. For all treatments, SBP returned to baseline by approximately 120 minutes after dosing. Mean SBP E_{max} was greater following *neffy* relative to Epinephrine IM ($P < .0001$ and $P < .0001$, respectively) but did not reach significance relative to EpiPen ($P = .0600$). Following repeated doses, change from baseline SBP was greater for both *neffy* treatments compared with EpiPen. Mean SBP E_{max} was significantly greater with *neffy* (R/R) and *neffy* (L/R) relative to EpiPen ($P = .0003$ and $P = .0004$, respectively). There was no significant difference in E_{max} between *neffy* (R/R) and *neffy* (L/R).

Treatment with a single dose of *neffy* resulted in an immediate increase from baseline DBP, followed by a decrease in DBP (Fig 2 and Table II). Both EpiPen and Epinephrine IM caused an immediate decrease from baseline DBP. The decrease was more pronounced following EpiPen and Epinephrine IM compared with *neffy*. Mean DBP E_{max} was significantly greater with *neffy* compared with EpiPen and Epinephrine IM ($P = .0475$ and $P = .0363$, respectively). Following repeated doses, both *neffy* treatments resulted in an initial increase from baseline DBP, followed by a return toward baseline. EpiPen resulted in an immediate decrease from baseline DBP that persisted until 120 minutes after dosing. Mean DBP E_{max} was significantly greater following *neffy* (R/R) and *neffy* (L/R) relative to EpiPen (L/R) ($P = .0198$ and $P = .0038$, respectively). There were no significant differences in mean DBP E_{max} between *neffy* (R/R) and *neffy* (L/R).

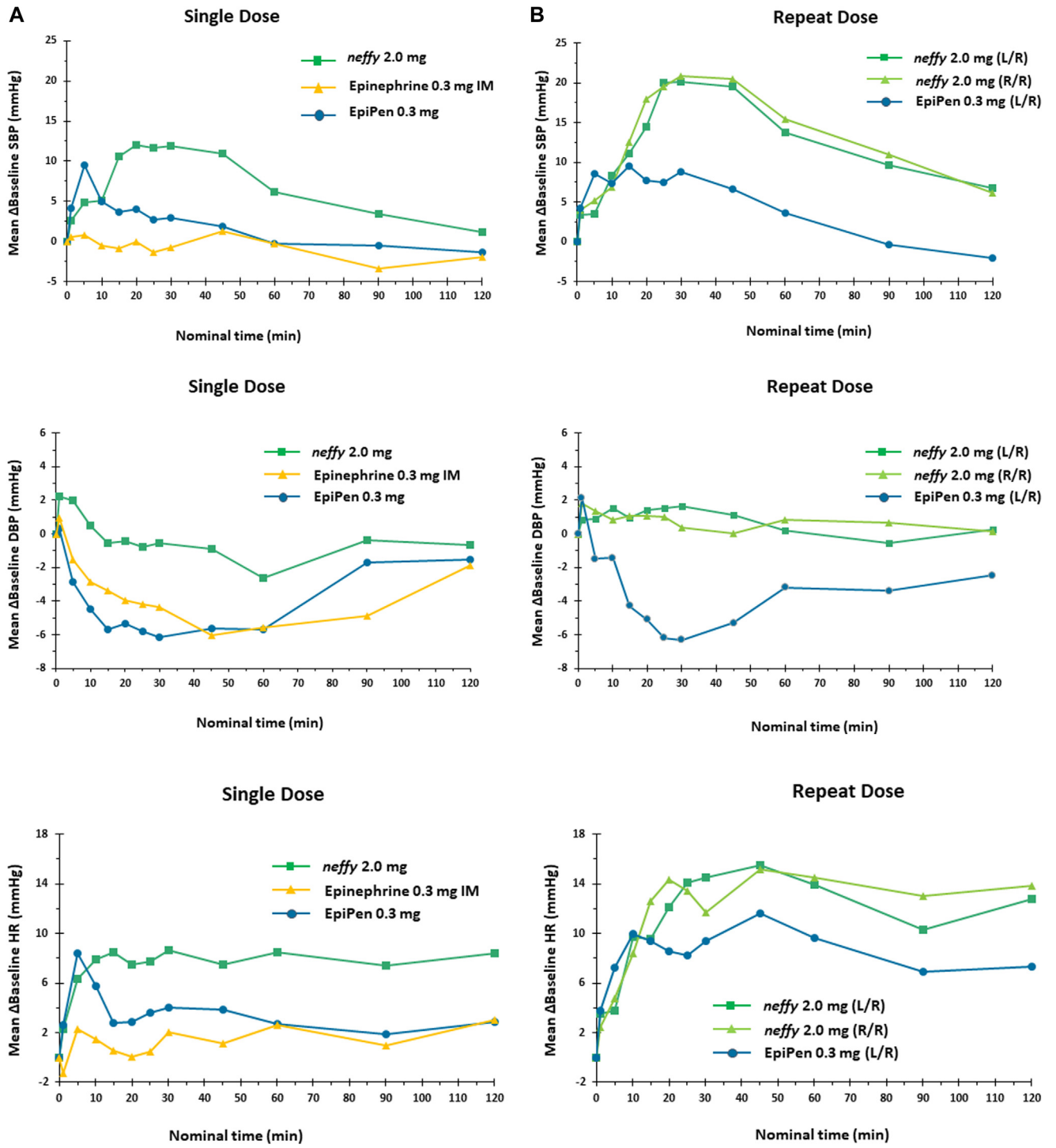

FIG 2. Mean change from baseline in SBP *(top)*, DBP *(middle)*, and HR *(bottom)* vs time. **(A)** Single dose. **(B)** Repeat dose.

Following a single dose, all treatments resulted in an increase from baseline HR (Fig 2 and Table II). The initial increase was followed by a decrease for both Epinephrine IM and EpiPen, whereas the elevation persisted throughout 120 minutes following *neffy*. In general, HR E_{max} was significantly greater following *neffy* compared with EpiPen and Epinephrine IM ($P = .0006$ and $P \leq .0001$, respectively). Following repeated doses, all treatments resulted in an increase from baseline HR. *neffy* (R/R) and *neffy* (L/L) were associated with more pronounced increases compared with EpiPen. Mean HR E_{max} was significantly greater following *neffy* (R/R) and *neffy* (L/R) relative to EpiPen ($P = .0011$ and $P = .0099$, respectively). There were no significant differences between *neffy* (R/R) and *neffy* (L/R).

TABLE II. Maximum pharmacodynamic effect (change from baseline) and time to maximum pharmacodynamic effect

Treatment	No. of subjects	Mean E_{max} (CV%)			Median T_{Emax} (min)		
		SBP (mm Hg)	DBP (mm Hg)	HR (beats/min)	SBP	DBP	HR
Single dose							
neffy 2.0 mg	42	23.6 (64.8)	8.10 (64.3)	17.3 (62.7)	25.0 (1.00-116)	13.0 (1.00-117)	19 (1.00-116)
EpiPen	42	18.2 (80.3)	5.62 (131)	12.3 (63.2)	9.0 (1.00-116)	10.0 (1.00-115)	10.0 (1.00-115)
Epinephrine IM	42	11.9 (81.0)	5.48 (145)	9.71 (87.1)	22.5 (1.00-116)	9.00 (1.00-115)	27.0 (1.00-117)
Repeat dose							
neffy 2.0 mg (L/R)	39	28.9 (47.0)	10.5 (71.2)	22.1 (55.0)	29.0 (2.00-116)	19.0 (1.00-115)	29.0 (1.00-116)
neffy 2.0 mg (R/R)	39	29.1 (46.0)	9.62 (83.5)	22.9 (44.3)	28.0 (6.00-85.0)	13.0 (1.00-118)	40.0 (1.00-116)
EpiPen (L/R)	42	19.1 (46.0)	6.31 (89.6)	17.4 (51.6)	15.5 (1.00-85.0)	5.00 (1.00-115)	24.5 (1.00-116)

CV%, Coefficient of variation.

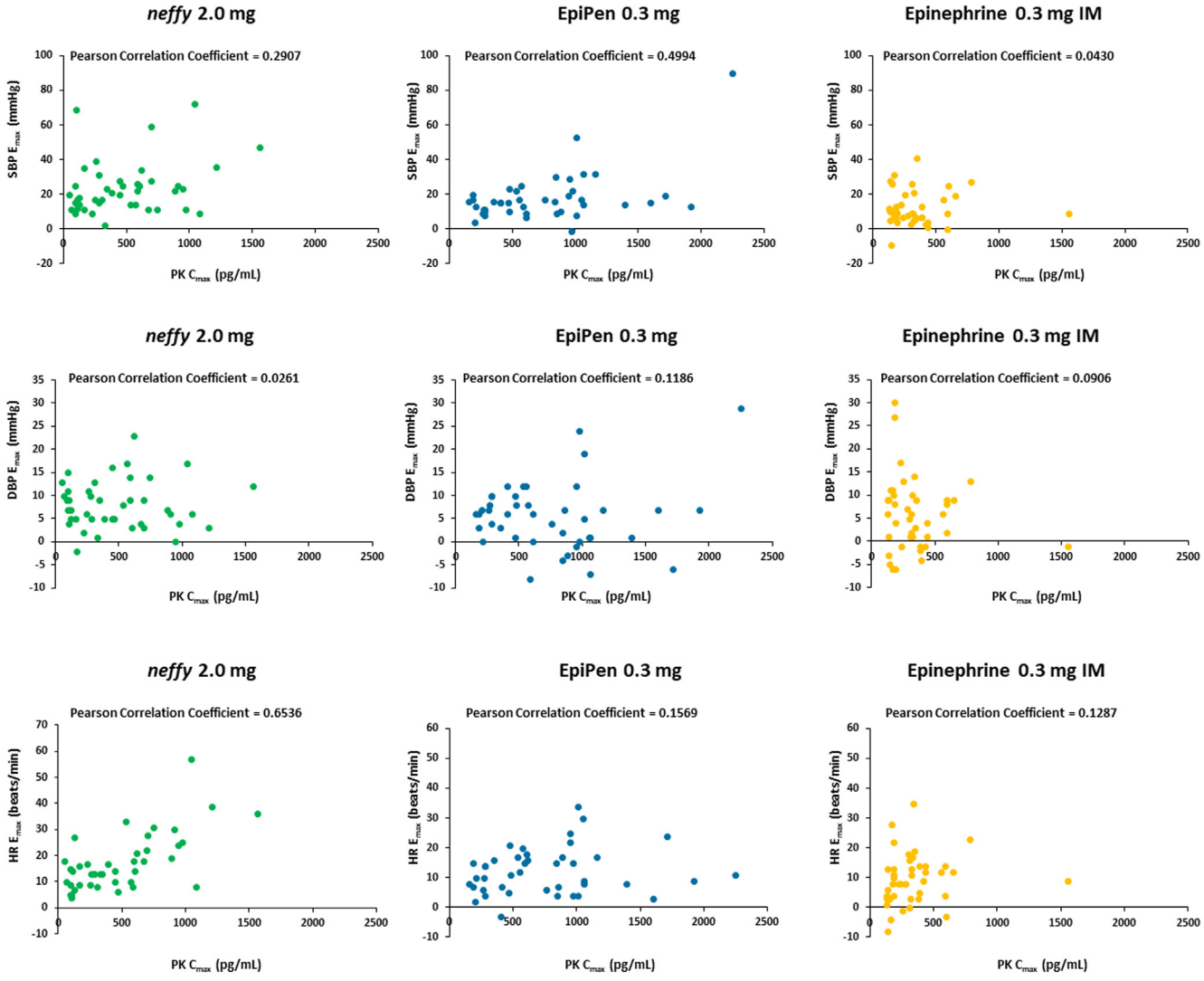

FIG 3. E_{max} vs C_{max} for SBP *(top)*, DBP *(middle)*, and HR *(bottom)*.

PK/PD relationship (E_{max} vs C_{max})

Scatter plots of individual relationships between E_{max} and C_{max} following single dose were generated for PD variables (Fig 3). Following *neffy*, SBP and HR increased as C_{max} increased. In contrast, following EpiPen and Epinephrine IM, SBP and HR were suppressed when C_{max} reached approximately 1500 pg/mL except for one subject whose SBP E_{max} was 90 mm Hg with C_{max} 2250 pg/mL following EpiPen.

Statistical analyses of the relationship between PK and PD were performed on single-dose data (Table III). *neffy* concentrations were strongly correlated with SBP (up to 45 minutes) and HR (up to 120 minutes), whereas correlations were observed

TABLE III. Statistical comparison between PD and PK

Treatment	Dependent variable*	Independent variable†	Time point	Slope	Adjusted r^2‡	*P* value§
SBP						
neffy 2.0 mg	SBP E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0122	0.0624	.0607
	ΔBaseline SBP (mm Hg)	Epinephrine concentration (pg/mL)	10	0.0253	0.1433	.0078
			15	0.0174	0.1005	.0231
			20	0.0176	0.1869	.0025
			30	0.0140	0.1456	.0080
			45	0.0163	0.1218	.0134
			60	0.0078	0.0208	.1790
			90	0.0091	−0.0061	.3917
			120	0.0106	−0.0101	.4472
EpiPen	SBP E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0148	0.233	.0007
	ΔBaseline SBP (mm Hg)	Epinephrine concentration (pg/mL)	10	0.0030	−0.0197	.6096
			15	0.0142	0.1079	.0249
			20	0.0140	0.1037	.0213
			30	0.0132	0.0573	.0691
			45	0.0066	−0.0155	.5443
			60	0.0109	−0.0130	.4946
			90	−0.0646	0.0247	.1611
			120	−0.0479	−0.0003	.3263
Epinephrine IM	SBP E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0017	−0.0231	.7846
	ΔBaseline SBP (mm Hg)	Epinephrine concentration (pg/mL)	10	0.0133	0.015	.2122
			15	0.0428	0.1286	.0113
			20	0.0114	−0.0107	.4515
			30	0.0269	0.0712	.0506
			45	0.0093	−0.0152	.5382
			60	−0.0106	−0.0087	.4266
			90	−0.0112	−0.0165	.5655
			120	−0.0302	−0.0069	.4009
DBP						
neffy 2.0 mg	DBP E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0004	−0.0243	.865
	ΔBaseline DBP (mm Hg)	Epinephrine concentration (pg/mL)	10	−0.0088	0.0462	.0918
			15	−0.0027	−0.0026	.3504
			20	−0.0032	0.0079	.2564
			30	−0.0040	0.0340	.1287
			45	−0.0036	0.0035	.2908
			60	−0.0049	0.0095	.2451
			90	−0.0135	0.0688	.0515
			120	−0.0176	0.0266	.1532
EpiPen	DBP E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0018	−0.0101	.4462
	ΔBaseline DBP (mm Hg)	Epinephrine concentration (pg/mL)	10	−0.0054	0.0099	.2478
			15	−0.0068	0.0167	.2103
			20	−0.0043	0.0027	.2982
			30	−0.0038	−0.0034	.3594
			45	−0.0053	−0.0126	.4875
			60	−0.0189	0.0234	.1668
			90	−0.0267	−0.0081	.4182
			120	−0.0363	0.0078	.2570
Epinephrine IM	DBP E_{max} (mm Hg)	C_{max} (pg/mL)	—	−0.0029	−0.0163	.5622
	ΔBaseline DBP (mm Hg)	Epinephrine concentration (pg/mL)	10	−0.0083	0.0165	.2039
			15	−0.0131	0.0236	.1660
			20	−0.0128	0.0298	.1433
			30	0.0002	−0.0256	.9834
			45	0.0001	−0.0250	.9851
			60	−0.0101	−0.0002	.3257
			90	−0.0006	−0.0250	.9697
			120	−0.0540	0.1282	.0114
HR						
neffy 2.0 mg	HR E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0194	0.42	< .0001
	ΔBaseline HR (beats/min)	Epinephrine concentration (pg/mL)	10	0.0264	0.2960	.0001
			15	0.0089	0.1030	.0217
			20	0.0070	0.0560	.0713
			30	0.0135	0.3174	< .0001

(Continued)

TABLE III. *(Continued)*

Treatment	Dependent variable*	Independent variable†	Time point	Slope	Adjusted r^2‡	*P* value§
			45	0.0226	1.0000	< .0001
			60	0.0342	0.6633	< .0001
			90	0.0499	0.6518	< .0001
			120	0.0652	0.4436	< .0001
EpiPen	HR E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0025	0.0011	.3132
	ΔBaseline HR (beats/min)	Epinephrine concentration (pg/mL)	10	0.0051	−0.0026	.3485
			15	0.0051	0.0082	.2607
			20	0.0160	0.1591	.0052
			30	0.0148	0.1746	.0034
			45	0.0046	−0.0149	.5315
			60	0.0405	0.2784	.0002
			90	0.0619	0.1665	.0043
			120	0.0157	−0.0125	.4862
Epinephrine IM	HR E_{max} (mm Hg)	C_{max} (pg/mL)	—	0.0043	−0.0076	.4103
	ΔBaseline HR (beats/min)	Epinephrine concentration (pg/mL)	10	0.0175	0.0422	.1046
			15	0.0316	0.1868	.0025
			20	0.0176	0.0812	.0395
			30	0.0060	−0.0149	.5238
			45	0.0118	0.0290	.1438
			60	0.0281	0.1876	.0024
			90	0.0099	−0.0143	.5190
			120	0.0427	0.0145	.2131

*PD change from baseline.
†PK concentration.
‡Adjusted r^2 of the regression.
§Significant difference defined *a priori* as $P < .05$.

only at 15 to 20 minutes following EpiPen and 15 minutes following Epinephrine IM. This discrepancy suggests a possible compensatory response that suppressed increases in SBP and HR following EpiPen and Epinephrine IM. PK/PD correlations were observed for DBP.

Safety results

All treatments were well tolerated, and all adverse events were mild. The greatest SBP E_{max} occurred following EpiPen, and the greatest HR E_{max} occurred following *neffy* (Fig 4).

DISCUSSION

neffy 2.0 mg was designed to have a PK profile within the range of currently approved epinephrine injection products. Because EpiPen is reported to have the fastest and highest C_{max}, and manual IM injection is reported to have the slowest and lowest C_{max},[4-6] both comparators were included in this study. The present study demonstrated that the PD profile of *neffy* is comparable to EpiPen and comparable to or better than Epinephrine IM injection, suggesting that *neffy* may be at least as efficacious as these approved products.

A single dose of *neffy* resulted in a mean C_{max} of 481 pg/mL, which falls between EpiPen (753 pg/mL) and Epinephrine IM (339 pg/mL) (Table I). Similarly, *neffy* had a median T_{max} value of 30.0 minutes, which was between EpiPen (7.5 minutes) and Epinephrine IM (45 minutes).

Following repeated doses of *neffy*, mean C_{max} increased to approximately 1000 pg/mL; no differences were observed when 2 doses were administered to the opposite naris (*neffy* [L/R]) versus the same naris (*neffy* [R/R]) (Table I). There was no significant difference in mean C_{max} values between repeated doses of EpiPen and repeated doses of *neffy*. Overall, epinephrine exposure was greater following *neffy* (R/R and L/R) relative to EpiPen. Repeat doses for *neffy* had dose proportionality, but not repeat doses for EpiPen.

Notably, despite having a lower C_{max}, *neffy* resulted in a more pronounced increase in DBP relative to EpiPen (Fig 2; Table II). One potential explanation for this is that IN administration bypasses the activation of high-affinity β_2 receptors in the skeletal muscle.[7] Epinephrine binds to β_1-, β_2-, α_1-, and α_2-adrenoceptors; however, the β-adrenoceptors have a higher epinephrine affinity relative to the α-adrenoceptors.[15] Epinephrine injection to the thigh, either via manual IM injection or an EAI, directly exposes skeletal muscle to 100% of the dose, resulting in rapid activation of the β_2 receptors in the vessels in the skeletal muscle. This β_2 activation promotes vasodilation, increases blood flow to the skeletal muscle, and reduces venous return, resulting in a rapid decrease in DBP,[16] and ultimately suppresses increases in SBP. In contrast, following *neffy*, epinephrine is absorbed through the capillaries in the nasal mucosa and enters systemic circulation. Once in the systemic circulation, the skeletal muscle is exposed to only 15% to 20% of the total dose based on the distribution of cardiac output at rest.[15] The marked decrease in DBP following EpiPen and Epinephrine IM observed in the current study is consistent with the differential receptor activation in response to the mode of administration.[7]

Similarly, *neffy* had the most robust and efficient effect on HR despite its lower C_{max} (Fig 2; Table II). β_1 receptors have higher affinity, and thus it is expected that the HR E_{max} would be strongly correlated with C_{max}. However, the HR increase was suppressed following EpiPen despite its higher C_{max} and shorter T_{max}. One potential explanation for this is that the rapid increase in plasma epinephrine concentrations, possibly due to partial intra-blood vessel administration, may have resulted in the activation of

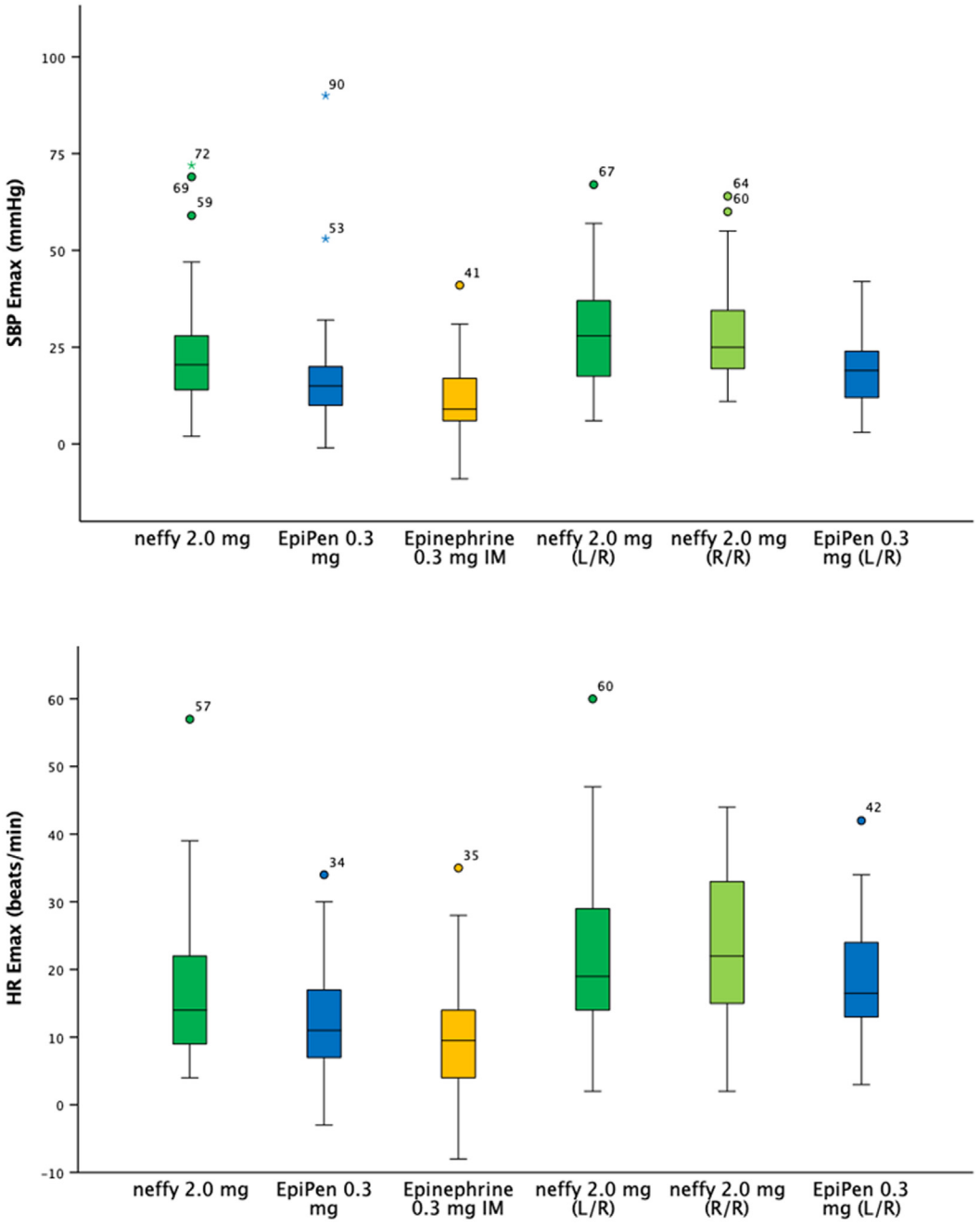

FIG 4. Box plots of SBP *(top)* and HR *(bottom)* E_{max}.

compensatory physiological mechanisms, including the carotid-aortic baroreceptor system, which regulates the dynamic monitoring and maintenance of BP and HR.[17] As a result, BP and HR measurements taken 1 to 2 minutes after EpiPen administration likely reflect these compensations. Therefore, when epinephrine levels increase rapidly and dramatically, such as seen in some subjects following EpiPen administration, the observed HR response may not be completely concentration dependent but rather a complex interaction involving both the rate/magnitude of increasing epinephrine concentration and adaptive homeostatic mechanisms. This hypothesis is further supported by the statistical analysis of the correlation between HR response and absorption of epinephrine (Table III), showing that for the first 15 minutes following EpiPen administration, there is no correlation between concentration and HR response. In contrast, following *neffy*, there is a consistent positive correlation between concentration and HR response, suggesting that the rate of increase in epinephrine levels following IN administration does not activate mechanisms involved in downregulating HR and BP.

Given that *neffy* is intended for use in patients experiencing severe allergic reactions/anaphylaxis, it is important to consider how the physiological changes associated with allergic reactions are impacted by the response to epinephrine treatment. During anaphylaxis, vasoactive amines such as

histamine are released from mast cells and basophils, resulting in vasodilation and hypotension.[14] A recent study suggested that peanut-induced allergic reactions resulted in reduced venous return due to significant fluid redistribution, which occurred regardless of reaction severity.[18] Under these conditions, it is likely that HR is already increased in response to decreased BP or venous return. However, additional increases in HR following administration of epinephrine may not be compensated for if the hemodynamics change due to allergic reactions were not resolved. Alternatively, epinephrine administration via IM injection may be augmenting mast cell mediator-induced vasodilation via the activation of the β_2 receptors in the thigh. This effect may be most pronounced after IM administration, when the high-affinity β_2 receptors are directly activated, and might persist until epinephrine concentrations are high enough to activate the lower-affinity α_1 receptors, which partially counterbalance the vasodilation.[16] Essentially, it is possible that even without the activation of compensatory mechanisms, the increase in BP resulting from the activation of β_2 receptors is suppressed by augmented vasodilation following IM injection into the skeletal muscle. Because IN administration does not directly activate skeletal muscle β_2 receptors, this degree of BP suppression is unlikely to be observed following *neffy*.

While *neffy* effectively increases BP and HR with minimal interference from β_2-mediated vasodilation or activation of compensatory mechanisms, it is important to note that there are PD ceilings, whereby, regardless of route of administration, increases in epinephrine concentrations no longer translate into increases in HR or BP. First, all cardiac and metabolic epinephrine actions are fully expressed at concentrations of approximately 1000 pg/mL.[19] Second, the vasoconstrictive effect of epinephrine, largely mediated by α_1 receptor activation, is attenuated by β_2-mediated vasodilation, resulting in a modulation of BP.[15] Third, increases in HR are limited by compensatory vagal discharge. These ceiling effects are key to the safety of IM and IN administration unless epinephrine is accidentally administered as an intra-blood vessel bolus.[20]

A limitation of this study is that because it was conducted in healthy volunteers, we cannot necessarily extrapolate the findings to patients experiencing severe allergic reactions. However, due to the high degree of variability in allergic and anaphylactic reactions as well as the relative rarity of these events, conducting studies in which anaphylaxis is induced is difficult and unethical. Therefore, it is important to evaluate the efficacy of epinephrine based on the available data with healthy volunteers and subjects with local allergic reactions as well as with preclinical data and vast clinical experience. While limitations are acknowledged, this report should serve to expand our understanding of epinephrine's mechanism of action.

The results of this study suggest that *neffy* has the potential to be an additional option for the first-line treatment of (type I) allergic reactions/anaphylaxis, particularly for patients/caregivers who are reluctant to use injectable epinephrine.

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Clinical implications: With its needle-free IN administration, *neffy* is expected to provide a safe and effective epinephrine delivery option, particularly for patients who delay or avoid dosing due to needle phobia.

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