Original Article

Bioavailability and Cardiovascular Effects of Adrenaline Administered by Anapen Autoinjector in Healthy Volunteers

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What is already known about this topic? Adrenaline autoinjectors used in anaphylaxis should have a sufficient needle length to reach the muscle. Their performance was analyzed using a novel combination of ultrasonography, adrenaline plasma level assays, and cardiovascular responses in human volunteers.

What does this article add to our knowledge? Subcutaneous as well as intramuscular adrenaline, delivered using an autoinjector with a relatively short needle, may ensure optimal bioavailability and cardiovascular response, even in overweight women. The analysis of early bioavailability parameters and cardiovascular response is necessary to assess the speed of action of the devices.

How does this study impact current management guidelines? The prediction of adrenaline autoinjector efficacy in anaphylaxis should be based on the combined assessment of ultrasonographic depot localization, the analysis of biphasic and parallel patterns of plasma adrenaline levels, and the cardiovascular responses in various categories of healthy volunteers.

BACKGROUND: The administration of adrenaline is a life-saving intervention for anaphylactic reactions. However, it has been questioned whether the needle length of the autoinjectors is sufficient to achieve genuine intramuscular delivery and optimal bioavailability.
OBJECTIVE: To assess the adequacy of Anapen, which has a relatively short needle length (10.5 mm), through a comparison of the depot localization, plasma pharmacokinetics, and cardiovascular responses of adrenaline delivered via Anapen versus a prefilled syringe with a 25.4-mm needle, which is generally used for intramuscular injections.
METHODS: This randomized, open-label, crossover study compared the impact of adrenaline administration at 2 sites in the thigh of 18 normal weight male volunteers, using either Anapen or the prefilled syringe; in addition, we studied the treatment of 12 overweight women with Anapen. The depot depth was measured by ultrasonography, plasma adrenaline level was evaluated by ultra performance liquid chromatography-mass spectrometry (UPLC-MS), and heart rates were measured using a Holter monitor.
RESULTS: Intramuscular injections were given with both devices at both thigh sites in nonobese men, but not in overweight women. Adrenaline levels showed a double peak, with parallel changes in the heart rate. The first peak, of potential vital importance in anaphylaxis treatment, occurred at approximately 10 minutes postinjection, with maximum concentration and area under the curve significantly higher with Anapen than with prefilled syringes; the magnitude of the second peak did not differ among the various conditions. Unexpectedly, in overweight women treated with Anapen, the magnitude of the first peak was similar to that observed in men, despite the injection being subcutaneous, and the overall bioavailability was enhanced.
CONCLUSIONS: Needle length and intramuscular injection are not absolute requirements for autoinjector efficacy, but the monitoring of injection location, biphasic adrenaline levels, and cardiovascular responses is important for the assessment of their therapeutic relevance in anaphylaxis. **(J Allergy Clin Immunol Pract 2018;6:1257-63)**

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Abbreviations used
AUC- Area under the curve
BMI- Body mass index
C_{max}- *Maximum concentration*
E_{max}- *Maximum effect*
HR- Heart rate
PK- Pharmacokinetic
SBP- Systolic blood pressure
T_{max}- *Time at the* C_{max}

Key words: *Anapen; Autoinjectors; Adrenaline pharmacokinetics; Anaphylaxis; Cardiovascular responses*

Anaphylaxis is a serious allergic reaction that occurs suddenly and requires immediate treatment because it may be fatal.[1-3] Prompt adrenaline injection is widely recognized as the primary medical therapy for this life-threatening condition.[4-8] Adrenaline decreases the release of histamine and other inflammatory mediators from mast cells and antagonizes all major symptoms of anaphylactic shock: it increases the heart rate (HR) and myocardial contractility (through β1-adrenoceptors), enhances peripheral vascular resistance (through α1-adrenoceptors), and induces bronchodilation with increased oxygen absorption (β2-adrenoceptors). Adrenaline has a very low oral bioavailability and intravenous self-administration is not easy, particularly in an emergency. Therefore, prompt intramuscular self-injection of adrenaline is recommended as first-line treatment for serious hypersensitivity reactions. However, individuals are often reluctant to use a syringe and expect difficulties in executing intramuscular injections. Thus, there is a medical need for a safe and easy-to-use autoinjector device to ensure prompt and adequate adrenaline blood levels and cardiovascular responses, at least equivalent to those of standard prefilled syringes. A pioneering pharmacokinetic (PK) study of an autoinjector in volunteers suggested that this was best achieved via intramuscular injection into the thigh.[9]

Anapen, a pen applicator marketed by Bioprojet (Paris, France), provides a dose of 0.3 mg adrenaline through a syringe and a 10.5 mm, 27G × 1/2-in needle. Although it has been used in Europe since 2003 for adrenaline delivery in anaphylactic shock, with an apparently good safety record, no clinical trial has yet been administered to check its performance.

Furthermore, the length of its needle, as well as that of autoinjectors with even longer needles, was suggested to be inadequate to achieve intramuscular delivery (and therefore obtain adequate responses), particularly in women or overweight individuals.[10-16]

The European Medicine Agency has recently recommended several measures to better ensure the appropriate patient use of adrenaline autoinjectors for severe allergic reactions and confirmed that intramuscular injection was the preferred route of administration.[17] In addition, the product manufacturers were asked to conduct PK/pharmacodynamics studies to better elucidate how adrenaline penetrates body tissues when administered through an autoinjector.

The aim of our study was to assess the efficacy and safety of Anapen, a device currently in use for urgent adrenaline delivery during anaphylactic shock. Thus, we compared the pharmacokinetics and pharmacodynamics of an injection of 0.3 mg or 0.5 mg adrenaline delivered via a standard syringe equipped with a 1-in (25.4 mm) 25G needle, traditionally used for intramuscular injection, or Anapen, in 2 sites of the thigh in normal weight men or in overweight women. Moreover, as the length of the Anapen needle has been proposed as possibly insufficient to obtain intramuscular delivery,[18,19] the injection depth was assessed by ultrasound imaging and compared with results obtained for syringes equipped with needles of greater length (~2.5-fold longer than that in the Anapen device). The bioavailability of adrenaline was also compared in each test scenario.

METHODS

Design

The trial was conducted according to the principles of Good Clinical Practice and in accordance with the ethical principles of Declaration of Helsinki. The appropriate approval was provided by the Ethics Committee of Lyon (Sud-Est II), France, and the French Drug Agency (Agence Nationale de Sécurité du Médicament et des produits de santé [ANSM], EudraCT-number 2014-004006-15). Written informed consent was obtained from all participants.

This trial was a single-center, randomized, open-label study to investigate the impact of 0.3 mg adrenaline intramuscular administration using various devices in 18 normal weight healthy men (body mass index [BMI], 18-26 kg/m^2) in a crossover manner and in 12 overweight, but otherwise healthy, women (BMI, 26-34 kg/m^2) (Table I).

The adrenaline injections were administered by a nurse under the supervision of the principal investigator. The role of the injection site was assessed in a crossover manner by using Anapen in normal weight men; as summarized in Table II, the injections were administered either in the middle anterolateral third of the thigh ("mid-injection," corresponding to the commercial device notice instructions) (group A) or in the inferior third anterolateral part of the thigh (group D).

To assess the influence of the needle length, the same men also received a "mid-injection" using a prefilled syringe equipped with longer needles (25G × 1-in [25.4 mm] [Reference: Becton Dickinson #300400 and 300600] vs 27G × 1/2-in [10.5 mm] in Anapen) (group B); the group of overweight women (group E) received the injection using the Anapen autoinjector in the inferior anterior third of the thigh.

Finally, in group C, 18 normal weight men were administered 0.5 mg of adrenaline by using a syringe equipped with a 25G × 1-in needle. All subjects were injected while lying down.

For groups A, B, and C, the injection was administered in the external middle third of the anterolateral part of the musculus quadriceps femoris, as per the Anapen information leaflet; that is, the tip of the device was pressed on the skin and the spring released by pressing the button. The middle third was defined by the middle third of the distance between the upper edge of patella and the middle of the inguinal line. The injection was administered strictly on the external anterolateral middle side of this area. For groups B and C, the entire length of the needle was inserted into the muscle, perpendicularly to the skin, with the instruction to expel the syringe content as rapidly as possible (maximum 1-2 seconds), to mimic the injection extent of the autoinjector. Between periods, the investigator was instructed to administer the injection in the same area of the thigh for each volunteer, to decrease possible variabilities associated with different anatomical localizations. Hence, the location of the first injection was marked on the skin for the subsequent treatments.

TABLE I. Demographic and baseline cardiovascular characteristics of healthy volunteers

Characteristic	Normal weight men (n = 18)	Overweight women (n = 12)
Age (y)	31.50 ± 9.23	33.25 ± 9.07
Height (cm)	179.17 ± 7.85	161.92 ± 6.52
Weight (kg)	75.03 ± 10.48	78.07 ± 7.91
BMI (kg/m^2)	23.28 ± 1.87	29.73 ± 1.89
SBP (mm Hg)	116.22 ± 10.11	107.92 ± 6.37
HR (bpm)	57.50 ± 11.51	65.33 ± 12.62

bpm, Beats per minute.
The data represent the mean ± SD.

For group D, the injection was administered in the external inferior third of the anterolateral part of the musculus quadriceps femoris, which was defined by the inferior third of the distance between the upper edge of patella and the middle of the inguinal line.

For group E, the injection was administered as instructed in the Anapen information leaflet, in the inferior anterior third of the thigh, as defined by the inferior third of the distance between the upper edge of the patella and the middle of the inguinal line.

For crossover treatments in men, the injection side (right or left thigh) was documented in the case report form (CRF) and the injection side, right or left, was altered between each consecutive treatment period.

To maintain the basal plasma concentrations of endogenous adrenaline at a low level, the subjects rested supine for a minimum of 1 hour before dosing in a quiet room without stimuli. For the quantification of plasma adrenaline, blood samples were collected from a catheter that was inserted at least 1 hour before dosing and transferred into polyethylene tubes containing dry ethylenediaminetetraacetic acid-dipotassium (EDTA-K2). Samples were obtained 30, 20, and 10 minutes predose, at 1 minute, and then every 2 minutes after the injection up to 12 minutes, and thereafter at 15, 20, 25, 30, 40, 50, 60, 90, 120, 150, 180, and 240 minutes postinjection. The blood samples were centrifuged at 4°C and stored at −80 °C until analysis.

Plasma adrenaline assay

The plasma levels of adrenaline were quantified by using a validated liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) method in the Bioprojet Biotech Laboratory in accordance with the guideline EMEA/CMPH/EWP/192217/2009 Rev. 1 and the rules of Good Laboratory Practice. The internal standard isoproterenol was added to plasma, and samples were extracted by using Oasis WCX (Waters, Guyancourt, France) solid-phase extraction plates. The eluted samples were separated on an Acquity UPLC HSS PFP column (Waters) at 30°C with a mobile phase of ammonium formate/acetonitrile and a run time of 5.1 minutes. The MS ion source (electrospray ionization [ESI]) was used in the positive multiple reaction monitoring (MRM) mode. The lower limit of quantification was 39.06 pg/mL. At the lower limit of quantification, the intraassay coefficient of variation (CV) was 9.0% and the interassay CV was 9.7%, respectively, for adrenaline; for other concentration levels, the intraassay and interassay CVs did not exceed 14.0%.

The following PK parameters were derived from the adrenaline plasma concentrations by using WinNonlin Phoenix V6.3 (noncompartmental analysis): C_{max1} and t_{max1} (the first maximum adrenaline plasma concentration observed within the first 20 minutes postdose and the corresponding time of occurrence), C_{max2} and t_{max2} (the second maximum adrenaline plasma concentration observed within the 0- to 240-minute postdose period and the corresponding time of occurrence), and $AUC_{0\text{-}20}/AUC_{0\text{-}240}$ (the areas under the concentration-time curve from time 0 to 20 minutes postdose to time 0 to 240 minutes postdose, calculated by using a linear trapezoidal method).

Cardiovascular changes

On the day of each dose administration, the hospitalization and subject conditions were highly standardized from 2 hours before administration to 2 to 3 hours after administration.

The measures to minimize stress-induced bias in adrenaline secretion included the insertion of the catheter for blood sampling at least 1 hour before dosing and at least 20 minutes before the first vital sign measurement and blood sampling and the maintenance of an unstimulating environment for volunteers, who had to remain lying in a quiet room and were not permitted to watch television or use computers and games.

The mean HR was evaluated using a 12-lead 1000-Hz Holter ECG in 1-minute time windows from 30 minutes predose to 2 hours postdose.

The systolic blood pressure (SBP) was measured sequentially before and after the administration of each study drug(s) by using an automatic sphygmomanometer.

STATISTICAL ANALYSIS

The PK parameters were calculated using noncompartmental methods and summarized using descriptive statistics. To conform with the study design (randomized crossover), the significance of the differences between groups was assessed by a mixed ANOVA, which considered the random patient factor, the fixed treatment factor, the fixed sequence factor, and the sequence-treatment interaction carryover effect. Given the small sample size, the sequence and carryover effects were removed from the analysis if their significance was more than .5. This analysis was conducted for all the differences, except for the comparison of normal weight men and overweight women (difference A − E), for which pairwise simple t tests were used (the multiple comparison of means was not corrected for type 1 inflation).

The cardiovascular changes were evaluated through the formal comparison of maximum effect (E_{max}) and tE_{max} of SBP and Holter-extracted HR by using an ANOVA model for crossover design, which included fixed effects for treatment, period, and sequence. The subjects were considered as a random effect. The estimates of treatment effects and the differences between treatments were calculated from their adjusted 90% CI for each E_{max}. The proportion of responders (defined as subjects with either an increase versus baseline of $\geq$10 beats per minute for HR or $\geq$10 mm Hg for SBP) was determined using a mixed logistic model design, which included fixed effects for treatment, period, and sequence. The comparison of the depth of injection between devices was analyzed using the same methods used for cardiovascular changes. The direct relationships between the adrenaline plasma level and HR and SBP were investigated for the development of appropriate PK/pharmacodynamic models.

RESULTS

Eighteen healthy normal weight men completed the crossover part of the study and 12 healthy overweight women completed

TABLE II. Skin-to-muscle distance and depth of the depot (mean ± SD) according to injection conditions

Injection condition	X	Y	Z	Skin-to-muscle distance (cm)	Depth of the depot (cm)	Difference (cm)
A/Anapen	M	Mn	0.3	0.59 ± 0.18	1.17 ± 0.50	−0.63 ± 0.34
B/Needle (1 in)	M	Mn	0.3	0.60 ± 0.17	2.42 ± 0.80	−1.80 ± 0.63
C/Needle (1 in)	M	Mn	0.5	0.59 ± 0.19	2.48 ± 1.16	−1.86 ± 0.89
D/Anapen	I	Mn	0.3	0.44 ± 0.10	1.08 ± 0.38	−0.66 ± 0.27
E/Anapen	I	Fo	0.3	1.58 ± 0.36	1.15 ± 0.36	0.44 ± 0.36

Fo, Overweight women; *I*, inferior; *M*, middle; *Mn*, normal weight men; *X*, injection site; *Y*, subjects; *Z*, adrenaline dose.
The values for each group are the mean ± SD. Conditions A, B, C, and D were for a single group of 18 normal weight subjects who received the indicated treatments in a crossover manner.

the study. A summary of the main patient characteristics is presented in Table I.

Ultrasonic imagery

Typical examples of ultrasonic images for a normal weight man and an overweight woman are presented in Figures 1 and 2, respectively. Skin-to-muscle distance and the depth of the fluid depot were determined on site by the operator and the mean values per treatment group are presented in Table II.

In normal weight men, the ultrasonic images indicated that the depth of the fluid depot (between −0.6 and 1.8 cm) was consistently greater than skin thickness, which showed maximum values of 1.03 cm at the thigh anterolateral middle third and 0.63 cm at the anterolateral inferior third. Correspondingly, the ultrasound review of visible fluid depots confirmed that the adrenaline injections were located intramuscularly in all cases except 1.

However, in overweight women, the skin thickness (mean value, 1.58 cm; range, 1.0-2.3 cm) was, as expected, greater than that in normal weight men, and 10 of 12 visible fluid depots were located subcutaneously, with only 1 in the muscle and 1 undetermined.

PK and cardiovascular data

The adrenaline concentrations before dosing were not quantifiable, that is, below 39.06 pg/mL, which confirmed the very low level of endogenous circulating adrenaline in resting adults.

The time profiles of the changes in adrenaline level and HR (Figure 3) were comparable under the different conditions: both presented a biphasic pattern that consisted of a first narrow peak, roughly covering the first 20 minutes after injection, with a slightly variable time at the C_{max} (T_{max}) (mean at ∼12 minutes, see Table I) closely followed by a broader peak with a second maximum at approximately 40 minutes postinjection. The 2 peaks were of similar size in most scenarios (except for group B, which used a syringe equipped with a 1-in needle) with regard to both plasma level and HR increases; the profiles of changes in SBP closely resembled those of HR (not shown). However, in overweight women injected with Anapen, the 2 peaks tended to merge in a single peak for both PK and HR changes.

Modeling the PK/pharmacodynamic relationships demonstrated the correlation of 2 variables: the relationship between adrenaline plasma concentration and HR followed a saturable E_{max} model defined by 3 parameters: E_{max}, EC_{50}, and E_0. As determined by the estimated parameters of the model, the maximum increase in HR was approximately 17 beats per minute ($E_{max} - E_0$). Half the maximum effect was obtained when the adrenaline plasma concentrations reached a median value of 388 pg/mL. The estimate of the change in HR at C_{max} was comparable with Anapen and a 1-in needle.

As the promptness of injection is of great clinical relevance, the characteristics of the first peak were quantitatively analyzed through the determination of T_{max}, C_{max}, and AUC values during the first 20 minutes after the injections. The bioavailability of adrenaline during this first period was then compared with the total bioavailability during a 4-hour postinjection period to estimate the corresponding AUCs and their ratios (Table III).

The total adrenaline availability measured by $AUC_{0\text{-}240min}$ did not differ significantly among the different injection conditions, except for overweight women administered 0.3 mg adrenaline via Anapen (condition E) and normal weight men administered 0.5 mg adrenaline (condition C): in both cases, the total AUC was approximately 40% higher than in the other conditions. Although C_{max2} did not differ significantly among the various conditions, this was not the case for C_{max1} and the changes were mirrored by those of the HR, which confirmed the relevance of the separate analysis of the 2 peaks.

In the comparison of groups A and B, significantly better availability of adrenaline was shown in the first 20 minutes postinjection after administration by the autoinjector compared with the syringe equipped with a 1-in needle; although T_{max} values did not differ significantly, C_{max1}, $AUC_{0\text{-}20min}$, and the ratio of the 2 AUCs were nearly twice as high. Compared with the proportion of responders (odds ratio given by the logistic model) over the first 20-minute postinjection period, the probability of success was over 4 times higher when adrenaline was administered via Anapen instead of the syringe with the longer needle. In contrast, there were no significant differences in any parameter when the Anapen device was used in either the median or inferior part of the thigh in normal weight men (Table III); the 2 HR profiles were almost identical when superimposed (Figure 3, right).

When Anapen was used to administer adrenaline to the overweight women, a slight, nonsignificant delay was observed in the first peak compared with normal weight men and a delayed decline in the plasma adrenaline level occurred, which was later mirrored by a prolonged HR increase.

Finally, the administration of a 66% larger dose of adrenaline (0.5 mg vs 0.3 mg) with a longer needle exerted a positive effect on the initial bioavailability, with an 80% increase in C_{max1} and an approximate doubling of $AUC_{0\text{-}20min}$ (compare A and C) in normal weight men.

Safety results

Overall, the study treatments were very well tolerated. A total of 20 treatment-emergent adverse events, all of mild or moderate

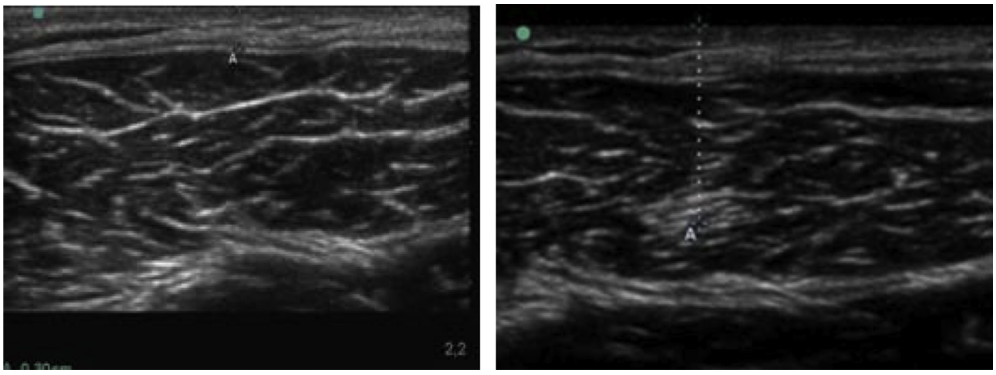

FIGURE 1. Ultrasonographic images of the skin of a normal weight male subject before (left) and after (right) injection of 0.3 mg adrenaline using the Anapen autoinjector. The dotted lines and letter A denote the skin limit (left) or the bolus localization (right).

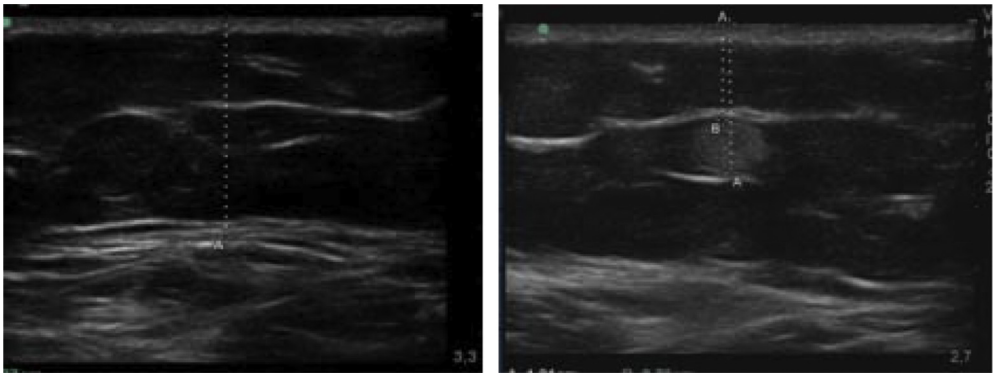

FIGURE 2. Ultrasonographic images of the skin of an overweight female subject before (left) and after (right) injection of 0.3 mg adrenaline using the Anapen autoinjector. The dotted line and letter A denote the skin limit (left) or the bolus localization (right).

severity, were reported in all treatment groups and their frequency was similar between treatments. Their nature and intensity reflected the expected pharmacological effects of adrenaline, such as palpitations, hand tremors, paresthesia, and headaches, which mostly occurred within the initial minutes after injection. The local tolerability was also excellent and no differences were observed either between treatments or volunteer populations.

DISCUSSION

In the present study, we assessed the potential efficacy of intramuscular adrenaline via the combined evaluations of skin-to-muscle distance, depot localization, hormone plasma bioavailability, and cardiovascular response in healthy volunteers of both sexes and different BMIs by using several injection devices and locations.

Our first conclusion was that, in normal weight healthy men, the adrenaline bolus reached the muscle, a prerequisite for efficacy,[9,17] when administered by the Anapen autoinjector, a device with a relatively short needle (0.394 in = 10.5 mm), or by a syringe equipped with a longer needle (1 in = 25.4 mm). This was true when the autoinjector was used in the middle or the inferior part of the thigh. In contrast, in women with a higher BMI and a greater skin depth, the use of the autoinjector in the inferior part of the thigh did not deliver adrenaline to the muscle.

We also concluded that the adrenaline pharmacokinetics and cardiovascular responses in both sets of data were well correlated in both the time course and relative amplitude. In particular, the changes in adrenaline plasma levels and HR clearly showed 2 successive peaks (Figure 3). The first peak occurred a few minutes after the injection (C_{max1} and E_{max} at ~10 minutes post-administration), lasted less than 20 minutes, and was followed by a larger peak, generally of similar height, which lasted up to 2 hours. The biphasic increase in adrenaline plasma level was consistent with data from previous trials of different devices, although it has not been previously analyzed in detail.[9] Conceivably, this biphasic response could result from an initial absorption of adrenaline, thereafter limited by local vasoconstriction, which is induced via the adrenergic alpha 1 receptor.

The height and duration of the initial peak are presumed to be highly relevant to the success of the treatment of anaphylactic shock, which is a life-threatening event that requires early intervention.[1-3]

The total bioavailability of adrenaline, assessed using $AUC_{0\text{-}240min}$ in plasma, did not vary significantly between subjects who were administered the same adrenaline dose (0.3 mg) irrespective of the device (Table III); furthermore, it appears to be in the same range as that reported with other autoinjectors equipped with longer needles than Anapen.[18] In contrast, the height and surface of the first adrenaline (or HR) peak was significantly higher after Anapen injections as compared with

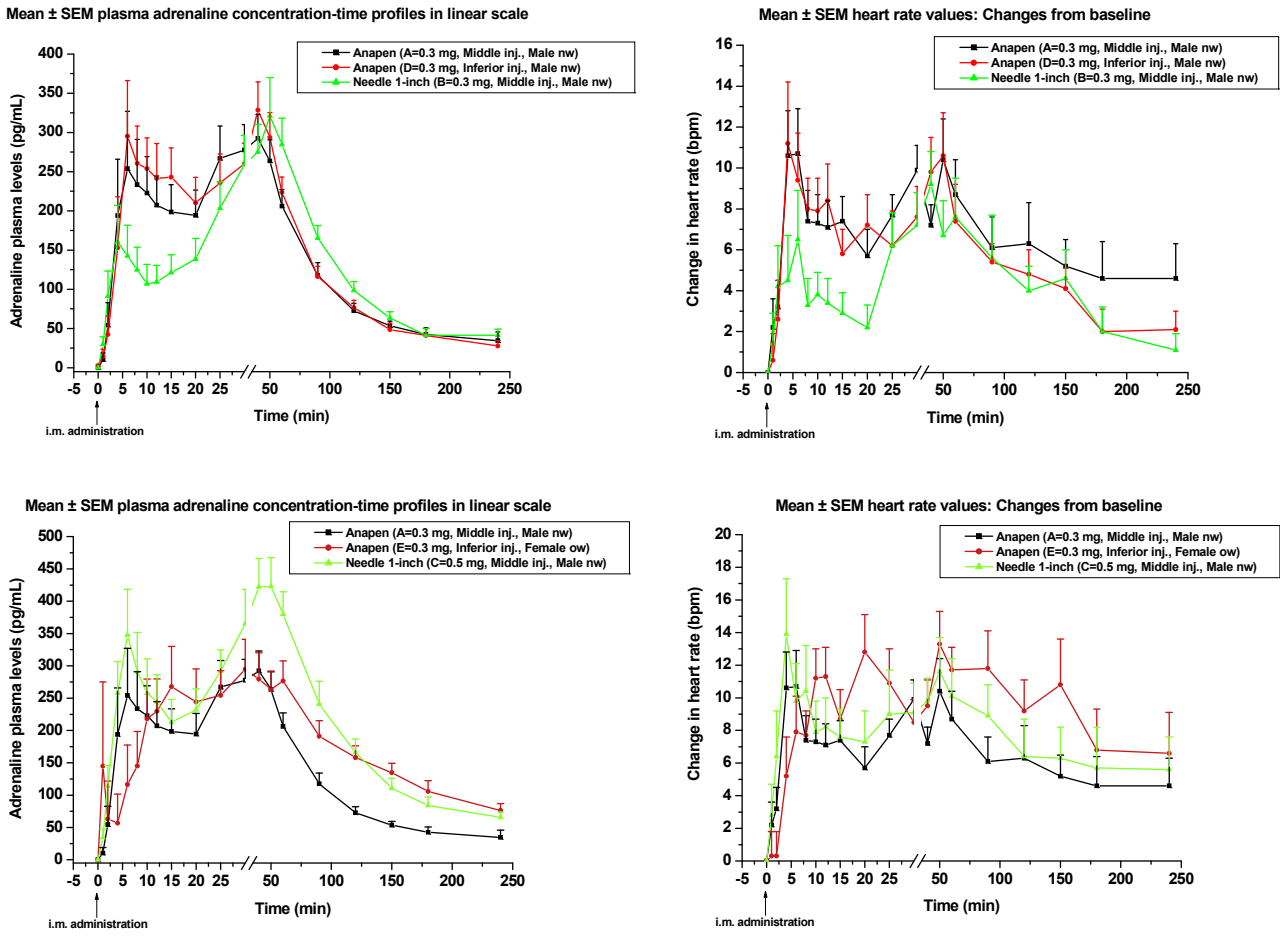

FIGURE 3. Changes in adrenaline plasma levels and HR according to various conditions.

injections with a syringe equipped with a longer needle (compare group B to group A or D); consequently, the ratio of initial to total exposure was twice as high with the autoinjector (7.7% compared with ~15%). In contrast, the magnitude of this early peak did not differ when Anapen injections were administered, either on the medial or on the inferior part of the thigh, but the muscle depth was inferior in the latter case. It is possible that the improved early delivery and action of adrenaline by the autoinjector may result in a higher speed and/or force of the bolus when expelled by a potent spring (rather than the slight difference [0.1 mm] in needle gauge). The probability of obtaining a cardiovascular response further to adrenaline injection was more than 4 times higher when adrenaline was administered using Anapen, instead of a syringe with a 1-in needle.

Recently, it was suggested that the autoinjector needle length might be inadequate for the intramuscular delivery of adrenaline in overweight women and that this might impact the morbidity and mortality due to anaphylaxis in this population.[13] Our study is apparently the first to assess the PK and efficacy of adrenaline administered via an autoinjector in women. Interestingly, in most overweight women (11 of 12) in whom the administration via Anapen failed to reach the muscle, the magnitude of the first peak in terms of T_{max}, C_{max}, or AUC did not differ from that observed in normal weight men and was immediately followed by a second larger peak, which resulted in a significantly higher total bioavailability than in normal weight men. This might be related to an impaired local or systemic inactivation of the hormone either by the metabolic system or by the uptake system in this group of subjects. This unexpected observation clearly suggested that needle length alone was insufficient to predict bioavailability and that intramuscular injections might not be a critical prerequisite for the successful administration by autoinjectors in anaphylaxis.

Collectively, our data confirmed that Anapen represents a valid therapeutic option in anaphylaxis, which was a contrasting conclusion to those based on needle length considerations or studies in ballistic gelatin (considered a valid tissue simulant) or porcine skin.[18-20]

Nevertheless, our study has some limitations. The major limitation is that data obtained in healthy volunteers cannot be extrapolated in a straightforward manner to subjects in a stage of anaphylactic shock in whom adrenaline has to oppose the effects of histamine and other mast-cell mediators. Furthermore, it was an open-label trial. In addition, several other groups of people, for example, children, who are at risk of intraosseous injection via overlong needles,[21] overweight men, subjects with a BMI of more than 34 kg/m^2, and normal weight women, were not studied. Furthermore, the effects of injections administered in the mid-thigh position instead of the recommended position in overweight women were not evaluated. Finally, the effects of Anapen at doses other than 0.3 mg were not studied.

TABLE III. Summary of the main PK parameters and comparison of changes according to conditions

Injection	X	Y	Z	T_{max} (0-20 min) (h)	C_{max} (0-20 min) (pg/mL)	C_{max} (0-240 min) (pg/mL)	$AUC_{0\text{-}20\ min}$ (h × pg/mL)	$AUC_{0\text{-}240\ min}$ (h × pg/mL)	AUC Ratio (%)
A/Anapen	M	Mn	0.3	0.21 ± 0.12	377.0 ± 298.1	449.6 ± 254.8	69.3 ± 54.0	459.2 ± 129.3	15.3 ± 11.0
B/Needle (1 in)	M	Mn	0.3	0.18 ± 0.13	222.6 ± 184.6	402.2 ± 208.2	38.5 ± 33.7	503.3 ± 201.2	7.7 ± 5.8
C/Needle (1 in)	M	Mn	0.5	0.15 ± 0.11	401.2 ± 266.9	578.5 ± 250.9	75.9 ± 52.6	777.2 ± 312.9	9.6 ± 5.7
D/Anapen	I	Mn	0.3	0.15 ± 0.08	353.9 ± 283.4	471.1 ± 241.0	68.4 ± 50.2	473.1 ± 139.4	14.5 ± 10.0
E/Anapen	I	Fo	0.3	0.24 ± 0.10	440.0 ± 416.3	533.6 ± 366.5	55.9 ± 44.5	677.7 ± 161.3	8.6 ± 7.1
A − B					154 (16 to 292) .043		30.8 (5.7 to 55.8) .032		7.6 (2.7 to 12.5) .011
D − B					131 (12 to 250) .042		29.9 (8.6 to 51.1) .015		6.7 (2.2 to 11.2) .012
A − D									
A − C								−318 (−424 to −211) .000	5.6 (1.6 to 9.6) .018
D − C								−304 (−420 to −188) 0.001	4.9 (1.7 to 8.1) .011
A − E								−218 (−308 to −128) .001	6.7 (0.3 to 13) .041

Fo, Overweight women; *I*, inferior; *M*, middle; *Mn*, normal weight men; *X*, injection site; *Y*, subjects; *Z*, adrenaline dose.
The values for each group are the mean ± SD. The conditions A, B, C, and D were for a single group of 18 normal weight subjects who received the indicated treatments in a crossover manner.
The differences between conditions A-B, E-B, etc are reported in the lower part of the table as the mean difference, (90% CI), and *P* value; empty spaces denote nonsignificant differences between the conditions.

In conclusion, our study highlighted the importance of the combined study of several parameters, such as the location of the injection and the depot, the plasma level of adrenaline, and the cardiovascular response, to assess the relevance of autoinjectors in anaphylaxis. The results indicated that neither needle length nor strict intramuscular localization constituted unique predictors of therapeutic success.

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