A preliminary study of intranasal epinephrine administration as a potential route for anaphylaxis treatment

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Summary

Background: **The intranasal (IN) administration of epinephrine could be an alternative route for anaphylaxis treatment. Although IN epinephrine absorption has been demonstrated in animals, such data in humans are still lacking.**

Objective: **To study the pharmacokinetics of IN epinephrine absorption in humans.**

Methods: **Each healthy adult (n=5) was administered IN saline, IN epinephrine at various doses (i.e., 0.3, 0.6, 1.25, 2.5 and 5 mg), and intramuscular (IM) epinephrine at 0.3 mg. Plasma epinephrine levels at baseline and various time points up to 120 minutes after administration were determined using high-performance liquid chromatography with electrochemical detection.**

Results: **Significant systemic absorption of epinephrine via IN route was observed only at the dose of 5 mg, and the absorption thereof was comparable to that of IM epinephrine; the average area-under-curve (AUC) values at 0-120 minutes for IN saline, IM epinephrine, and 5 mg IN epinephrine were 0.3, 18.3, and 19.4 ng.min/mL, respectively. In addition, the peak epinephrine concentrations and the time to reach them were also not significantly different between IM and 5-mg IN epinephrine; the corresponding values (mean ± SD) were 309 ± 88 pg/mL and 67 ± 43 min for IM epinephrine, and 386 ± 152 pg/mL and 70 ±17 min for 5 mg IN epinephrine.**

Conclusion: **This preliminary study showed that epinephrine can be significantly absorbed via the IN route in humans. However, it requires a higher IN dose (5 mg) than the usual IM dose (0.3 mg) to achieve comparable systemic epinephrine absorption.** ***(Asian Pac J Allergy Immunol 2016;34:38-43)***

Keywords: *epinephrine; pharmacokinetics; administration, intranasal; injections, intramuscular; anaphylaxis; therapeutics*

Introduction

Systemic anaphylaxis is a severe IgE-mediated reaction, which is commonly triggered by foods, drugs, or insect stings. The incidence of anaphylaxis is approximately 50-2,000 episodes per 100,000 persons or a lifetime prevalence of 0.05-2%.[1] In severe cases, anaphylaxis can result in fatality. Epinephrine is the drug of choice for the initial treatment of anaphylaxis. Failure to administer epinephrine promptly has been identified as the most important factor contributing to death in patients with systemic anaphylaxis. Therefore, it is recommended that patients with a history of severe anaphylactic reactions or the caregivers thereof have epinephrine readily available for an intramuscular (IM) injection as a first aid treatment.[2]

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Although epinephrine is available as an auto-injector (e.g., Epi-PEN®, Jext®) for convenient use in case of severe anaphylactic attacks, they are often underused for various reasons. First, epinephrine auto-injectors are not readily available to many patients, particularly those in developing countries, because of their relatively high cost.[3, 4] Second, even with auto-injectors, many patients are reluctant to use them, probably due to a lack of confidence in their use or a fear of needles.[5] In addition, proper instruction, repeated training and practice are also needed for the correct use of auto-injectors.[6]

To overcome the above drawbacks, alternatives to epinephrine auto-injectors have been investigated, e.g., epinephrine administered via inhalation, sublingual, or intranasal (IN) routes. Epinephrine inhalation has been shown to be ineffective when used in children because the number of epinephrine inhalations required and the bad taste of the inhalations make most children unable to inhale sufficient epinephrine to achieve the therapeutic concentration rapidly and significantly.[7] A study in rabbits showed that epinephrine administered via a sublingual route can be systemically absorbed at the equivalent amount to IM epinephrine.[8] However, the equivalent sublingual dose (40 mg) was about 100-fold higher than the usual IM dose (0.3 mg), but data in humans are still lacking.

An IN route is another potential method for drug administration because the nasal mucosa is a highly vascularized and permeable tissue with excellent absorption capability. Previous studies in a dog model have demonstrated that epinephrine administered via IN is readily absorbed into the systemic circulation.[9, 10] In humans, systemic epinephrine absorption has also been reported during topical application for endoscopic sinus surgery.[11] These findings suggest that an IN route could be used as an alternative route for epinephrine administration in anaphylaxis treatment. In this preliminary study, the pharmacokinetics of epinephrine administered via an IN route in a small group of healthy adult subjects was investigated and compared with the usual IM epinephrine to determine its feasibility in humans.

Methods

Materials and reagents

Epinephrine bitartrate, 3,4-dihydroxybenzylamine hydrobromide (DHBA), diphenylboric acid ethanolamine complex (DPBEA), ethyleneglycol-bis (2-aminoethylether) tetraacetic acid (EGTA), and sodium acetate were obtained from Sigma -Aldrich (St. Louis, MO, USA). Tetra-n-octylammonium bromide (ToABr) was from Fluka (Glossop, UK). Octanol, methanol, *n*-heptane, ammonia, ammonium chloride, and glacial acetic acid were from Fisons (Loughborough, UK).

Subject selection

The study was approved by the Institutional Ethics Committee of the Faculty of Medicine Siriraj Hospital, Mahidol University. Five healthy volunteers, 2 males and 3 females, aged 20-26 years old, were enrolled into this study. The subjects were deemed healthy based on their medical history, physical examination, ECG, and laboratory investigations including urinalysis, and routine hematological and biochemical tests. The subjects were excluded if they had any history of cardiovascular, thyroid, or central nervous system disorder or if they smoked or used any medications or recreational drugs. Prior to participating in the study, they were informed about the details of the study and gave their informed consent.

Study design and outline

The subjects were given each of the following treatments: 1) IN spray of saline solution as a negative control, 2) IM injection of epinephrine USP 1:1,000 at 0.3 mg (0.3 mL) in the lateral part of the right thigh as a positive control, and 3) IN spray containing epinephrine bitartrate at 0.3, 0.6, 1.25, 2.5, and 5 mg. Before the study, they were asked to abstain from methylxanthine-containing diets (e.g., chocolate, cocoa, or cola) for at least 24 hours. On the study day, they had an indwelling venous catheters inserted for blood sample collection and were continuously monitored for blood pressure, heart rate, and ECG throughout the entire study period. The venous blood samples were collected from the indwelling venous catheter for plasma epinephrine measurement 15-30 minutes before drug administration (as baseline or time 0) and at 5, 10, 15, 20, 30, 45, 60, 90 and 120 minutes thereafter. At each time point, 10 mL of whole blood was collected in a heparin tube containing 75 µL of the EGTA-glutathione solution (9.5% EGTA and 3% glutathione, pH 6-7). The sample was put on ice and processed within 1 hr after collection by spinning down at 1,600 g for 10 min at 4°C to collect plasma. The plasma was stored as 600-µL aliquots and kept at -80°C until analysis.

Because the study procedures in each session required a long period of inactivity, indwelling

venous catheterization, and continuous monitoring, there was a risk that this could cause stress to the subjects and affect the plasma levels of epinephrine due to an increased release from the endogenous source, particularly if the experiments using different epinephrine doses were performed consecutively in the same day. Therefore, the tests were done on separate days to avoid the above confounding factor and allow subjects to recover from the previous treatment.

High-performance liquid chromatography (HPLC) analysis of plasma epinephrine concentrations

Plasma epinephrine was extracted using a liquid/liquid extraction procedure followed by HPLC analysis with electrochemical detection, as described by Forster et al.[12] with slight modifications. Before analysis, the frozen plasma aliquot was thawed and centrifuged at 1,200 g for 5 min, and 475 µL of the supernatant was transferred to a 2-mL microcentrifuge tube. To the plasma sample, 25 µL of 10 nM DHBA in 400 mM acetic acid and 250 µL of ammonia buffer (2 M ammonium chloride adjusted to pH 8.8 with 2 M ammonium hydroxide containing 2 g/L DPBEA) were added and mixed well before adding 1 mL heptane mixture (heptane containing 3.5 g/L ToABr and 10 mL/L octanol). The sample was mixed by vortexing for 2 minutes followed by centrifugation at 1,200 g for 1 minute to separate the layers. The top organic layer (800 µL) was transferred to a 1.5-mL microcentrifuge tube, to which 380 µL octanol and 40 µL of 400 mM acetic acid were added. The acid infranate was collected into a 0.5-mL microcentrifuge tube for HPLC analysis.

For chromatographic analysis of epinephrine, a Waters HPLC system (Milford, MA, USA) was used; the system consists of a Waters 2795 separations module (Alliance HT), a Waters 2465 electrochemical detector, and a Waters Symmetry Shield RP18 column (150 x 2.1 mm, 3.5 µm). The mobile phase consisted of acetate buffer (110 mM sodium acetate pH 5.1, 5 mM sodium octane sulfonate, 1 mM EDTA) and methanol at a ratio of 90:10. It was filtered through a 0.45-µm Millipore filter and degassed under vacuum before use. The mobile phase was pumped isocratically at a flow-rate of 0.25 mL/minute. Twenty microliters of the sample was injected onto the HPLC column, and the effluent was monitored using an electrochemical detector with the potential set at 0.85 V with an Ag/AgCl reference electrode. The separation was done in an oven set at 35°C to overcome temperature fluctuations within the laboratory.

Preparation of intranasal epinephrine spray and administration

Intranasal epinephrine spray was freshly prepared before use from the Pharmacy Department at Siriraj Hospital by dissolving a specified amount of epinephrine bitartrate in normal saline solution and filter-sterilizing the solution. Each administration of the nasal spray device dispensed 125 ± 4 µL of spray volume containing epinephrine at 0, 0.3,0.6, 1.25, 2.5 or 5 mg (coefficient of variation of device output = 4%). Before IN administration, the volunteers underwent nasal irrigation with saline solution. Then, the tip of the device was inserted into the nasal cavity adjacent to the inferior turbinate and the drug was administered. The procedure was similar to that recommended for intranasal corticosteroid usage.

Data calculation and statistical analysis

To determine the rate and extent of epinephrine absorption, the area under the plasma concentration versus time curve at 0-120 minutes ($AUC_{0\text{-}120\ min}$) was calculated using the linear trapezoid method; in this calculation, the plasma epinephrine concentration at each time point was subtracted from the baseline plasma epinephrine to obtain the baseline-adjusted $AUC_{0\text{-}120\ min}$. A non-parametric Mann-Whitney U test was used for statistical analysis, and the differences were considered statistically significant at p value < 0.05.

Result

As shown in Figure 1, IM epinephrine resulted in significant systemic absorption over the period of 120 minutes compared with IN saline; the corresponding $AUC_{0\text{-}120\ min}$ (mean ± SD) values were 18.3 ± 9.3 and 0.3 ± 1.2 ng.min/mL, respectively ($p < 0.05$). For IN epinephrine, significant systemic absorption was observed only at the dose of 5 mg with the $AUC_{0\text{-}120min}$ of 19.4 ± 12.1 ng.min/mL ($p < 0.05$, compared with the IN saline group). The IN absorption of epinephrine at this dose was comparable to that of IM epinephrine and was not statistically different.

The pharmacokinetics parameters of IN epinephrine at 5 mg were also not significantly different from those of the IM epinephrine group; the plasma epinephrine concentrations at baseline (C_{basal}) and maximum (C_{max}) as well as the time to reach C_{max} (T_{max}) are as shown in Table 1. The average C_{max} for the IM and IN groups were 309 and 386 pg/mL, and the average T_{max} were 67 and 70 minutes, respectively. The plasma epinephrine concentration-time curves of both IM and 5-mg IN epinephrine groups are shown in Figure 2, with that of the IN saline group as a control.

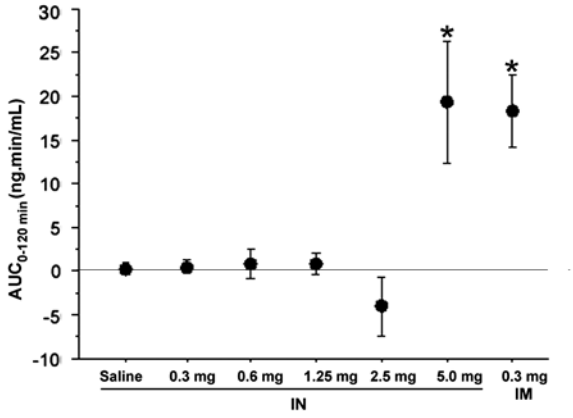

Figure 1. The area under the plasma concentration versus time curve at time 0 – 120 minutes ($AUC_{0\text{-}120\ min}$) of epinephrine absorption at different dosages and routes of administration. The plots are shown as mean ± SEM. An asterisk indicates a significant difference compared with the IN saline ($p < 0.05$, Mann-Whitney test).

Table 1. Pharmacokinetics parameters of epinephrine absorption via intranasal and intramuscular routes

Mean ± SD	Intramuscular (IM) Epinephrine 0.3 mg	Intranasal (IN) Epinephrine 5 mg
$C_{baseline}$ (pg/mL)	35 ± 23	8 ± 6
C_{max} (pg/mL)	309 ± 88	386 ± 152
T_{max} (min)	67 ± 43	70 ± 17
$AUC_{0\text{-}120\ min}$ (ng.min/mL)	18.3 ± 9.3	19.4 ± 12.1

$C_{baseline}$, baseline plasma epinephrine concentration; C_{max}, maximum plasma epinephrine concentration; T_{max}, time at which maximum plasma epinephrine concentration was achieved; $AUC_{0\text{-}120\ min}$, area under the plasma concentration versus time curve (t = 0 to 120 minutes)

During the course of the study, transient tremor was observed in one subject and palpitation in two subjects. There were increases in heart rate, and diastolic and systolic blood pressures at T_{max} in most subjects but no correlation was found between these symptoms and the plasma concentrations of epinephrine. No serious adverse effects were observed in the subjects after epinephrine administration.

Discussion

Nasal mucosa is a promising site for drug absorption into the systemic circulation due to its rich vasculature and high permeability. Examples of drugs administered through this route range from small compounds such as anti-inflammatory glucocorticoid,[13] analgesics,[14] and antiepileptics[15] to larger macromolecules such as polypeptides and proteins.[16,17] In emergency situations, IN delivery is a convenient route for drug administration and thus could be considered an alternative route of epinephrine administration for anaphylaxis treatment, particularly in situations where epinephrine auto-injectors may not be readily available, or the patients are reluctant to use them.

In this study, significant systemic absorption of epinephrine via the IN route was observed only at the dose of 5 mg. Although the pharmacokinetic parameters of IN epinephrine at this dose (i.e., C_{max}, T_{max}, and $AUC_{0\text{-}120min}$) were not significantly different from those of IM epinephrine at the usual dose of 0.3 mg, the plasma epinephrine levels of the IM group seemed to increase early (as early as 5-10 minutes) and remained higher than the baseline over 120 minutes after administration (Figure 2), which is similar to the findings from the previous studies.[8, 18, 19] In the IN group, plasma epinephrine seemed to elevate more slowly (about 15-20 minutes after administration), reached a peak at around 60 minutes, and then declined to the baseline level at 120 minutes (Figure 2). The somewhat slower epinephrine absorption through the IN route is not unexpected, as the drug has to diffuse across the nasal epithelial barrier to reach the systemic circulation. This could also explain a much higher IN dose (5 mg) required to achieve systemic absorption comparable to the IM route at 0.3 mg. The results here are in agreement with those from a similar study by Rawas-Qalaji et al.,[8] showing that epinephrine as high as 40 mg is required for sublingual administration to obtain similar plasma epinephrine concentrations to those of the 0.3-mg IM administration.

Another issue that could affect IN absorption is the nasal spray formulation. In this study, the epinephrine spray was simply prepared fresh in normal saline before use. However, for practical reasons, the spray should be prepared using the type of buffer, pH, and antioxidants that can make epinephrine stable and active for a reasonable period

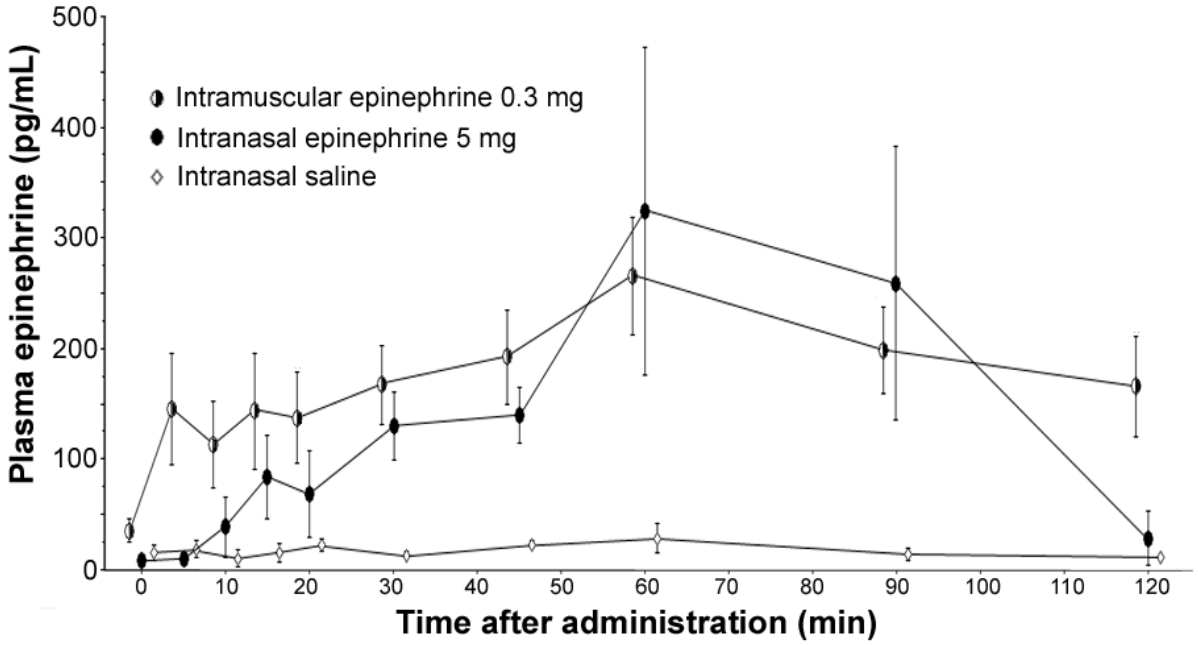

Figure 2. Plasma epinephrine concentration versus time plots after administrations of IN saline, IN epinephrine at 5 mg, and IM epinephrine at 0.3 mg. The plots are shown as mean ± SEM.

of time so that the nasal spray can be carried and used by the patients during anaphylactic attacks. In addition, drugs with vasodilatation effect, e.g. alpha-adrenergic receptor antagonists such as phentolamine, might be added to the formulation to counter the vasoconstriction effect of epinephrine, which prevents its own systemic absorption. Using bile salt or surfactant as a vehicle might also promote the nasal absorption of epinephrine because they have been shown to work well in a dog model.[9, 10, 20]

Because this study was performed in healthy subjects, the results may not reflect the absorption of the drug during an anaphylactic situation whereby the blood supply to the nose is compromised. However, studies in a dog model have shown that IN epinephrine is efficiently absorbed through nasal mucosa and can rescue dogs with induced ventricular fibrillation during CPR; the effects are comparable to standard-dose IV epinephrine.[9, 10] Thus, comparable results might also be expected in humans.

Although the feasibility of epinephrine administered via IN in humans has been demonstrated in this study, the results are still preliminary because the pharmacokinetics were studied in a small group of healthy subjects. Therefore, further work is still needed before IN epinephrine delivery can be used for anaphylaxis treatment. For example, the study should be repeated in a larger sample size and at the IN doses of 5 mg or higher to confirm the reproducibility of these findings and to obtain the optimal IN doses. In addition, different epinephrine formulations for nasal spray should also be investigated to discover one with good *ex vivo* and *in vivo* stability and which can achieve better and faster systemic absorption.

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Conflict of interest

The authors declare that there are no conflicts of interest in the research.

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