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Epinephrine Absorption after Different Routes of Administration in an Animal Model

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ABSTRACT: *Background*: the administration of epinephrine is the most important initial treatment in systemic anaphylaxis. *Purpose*: we wanted to determine the relationship between the route of epinephrine administration and the rate and extent of epinephrine absorption. *Methods*: in a prospective, randomized, five-way crossover study in rabbits, we measured plasma epinephrine concentrations before, and at intervals up to 180 min after epinephrine administration by intramuscular or subcutaneous injection, or by inhalation, with intravenous epinephrine and intramuscular saline as the positive and negative controls, respectively. *Results*: maximum plasma epinephrine concentrations were higher, and occurred more rapidly, after intramuscular injection than after subcutaneous injection or inhalation, and were 7719 ± 3943 (S.E.M.) pg/mL at 32.5 ± 6.6 min, 2692 ± 863 pg/mL at 111.7 ± 30.8 min and 1196 ± 369 pg/mL at 45.8 ± 19.2 min, respectively. Intravenous injection of epinephrine resulted in a plasma concentration of 3544 ± 422 pg/mL at 5 min, and an elimination half-life ($t_{1/2}$) of 11.0 ± 2.5 min. In the saline control study, the endogenous epinephrine concentration peaked at 518 ± 142 pg/mL. *Conclusion*: in this model, absorption of epinephrine was significantly faster after intramuscular injection than after subcutaneous injection or inhalation. The extent of absorption was satisfactory after both intramuscular and subcutaneous injections. Neither the rate nor the extent of absorption was satisfactory after administration by inhalation.

Key words: epinephrine; adrenaline; anaphylaxis; intramuscular; subcutaneous; inhalation; rabbit

Introduction

Since the early 20th century, epinephrine has been considered to be the medication of choice in the initial treatment of systemic anaphylaxis. In descriptive reviews [1–3], book chapters [4–12], and position statements [13–16], an astonishing variety of authoritative recommendations for epinephrine administration have been made, based on anecdotal experience. There is no published prospective, randomized, controlled, comparative study of the various routes of epinephrine administration, doses, dose intervals or plasma concentrations achieved.

We hypothesized that the administration of epinephrine by intramuscular injection would provide peak plasma concentrations more rapidly than administration by either subcutaneous injection or inhalation from a pressurized meter dose inhaler. We tested this hypothesis in a prospective, randomized, single-dose, placebo-controlled, five-way crossover study in an animal model, using intravenously injected epinephrine as the positive control and intramuscularly injected normal saline as the negative control.

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Materials and Methods

The research was conducted according to current guidelines published by the Canadian Council on Animal Care [17], and was approved by The University of Manitoba Fort Garry Campus Protocol Management and Review Committee. Six New Zealand white rabbits, mean weight 4.2 ± 0.1 kg, were studied. Before and between studies, each rabbit was kept individually in a metal cage with a wire floor to reduce coprophagy. Food and water were supplied *ad libitum*. During each study, rabbits were kept in a restrainer cage (Nalgene, Rochester, NY, USA).

For the intramuscular, subcutaneous or intravenous injection of epinephrine, Adrenalin® (1 mg/mL) (Parke-Davis, Scarborough, Ontario, Canada) [18] was used. In the case of intramuscular or subcutaneous injection, 0.3 mL Adrenalin® 1 mg/mL was diluted to 1 mL with sterile 0.9% sodium chloride; then 0.1 mL (0.03 mg) was injected intramuscularly or subcutaneously into the lateral aspect of the right thigh. In the case of inhalation, the Bronkaid Mistometer® (0.275 mg at the valve; Sanofi Winthrop, Markham, Ontario, Canada) epinephrine formulation was used. Ten puffs were administered into an AeroChamber® with Infant Mask (Trudell

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Medical, London, Ontario, Canada) [18] at a rate of 1 puff/30 s (10 puffs/5 min). During this time, the rabbit's neck was extended slightly, the clear plastic mask was applied gently but firmly to the face, and the animal was observed to be breathing regularly and quietly as evidenced by condensation on the mask. As a positive control, 0.5 mL Adrenalin® 1 mg/mL was diluted to 1 mL and a dose of 0.1 mL (0.05 mg) was injected into an ear vein by slow push over 30 s. As a negative control, 0.3 mL of normal saline was injected intramuscularly.

In each rabbit, on each of 5 study days at least 2 weeks apart, a 1.5 mL blood sample for measurement of plasma epinephrine was obtained from an indwelling catheter in an ear artery, before and 5, 10, 15, 20, 30, 40, 60, 90, 120 and 180 min after the administration of epinephrine or saline. At baseline on each study day, and at all the above time intervals after the intramuscular injection of saline, endogenous plasma epinephrine concentrations were measured.

Plasma Epinephrine Concentrations

Blood samples were centrifuged at 1600g, 4°C. Plasma was transferred into appropriately labelled polypropylene tubes with screw caps, frozen promptly in an upright position, and stored at −20°C until analysis.

Plasma epinephrine concentrations were measured using a reverse phase high performance liquid chromatography (HPLC) system with electrochemical (EC) detection [19]. The extraction efficiency was 70–80%. The lower limit of detection of epinephrine using this assay was 5 pg (0.025 nM). Calibration curves were linear over the range 25–1000 pg (0.125–5 nM) with a coefficient of variation of 10 and 3%, respectively.

Data Analysis

After epinephrine administration by the intravenous route, pharmacokinetic parameters were calculated from the plasma epinephrine concentration versus time plots using standard pharmacokinetic equations and the computer program WinNonlin (Scientific Consulting, Apex, NC, USA). Plasma concentrations at 5 min were recorded as the maximum concentrations after iv injection. After epinephrine administration by the intramuscular or subcutaneous routes or by inhalation, and after saline administration, only the maximum epinephrine concentration (C_{max}), the time of maximum epinephrine concentration (T_{max}), and the area under the concentration versus time curve (AUC) could be calculated, as variability of C_{max} and T_{max} values did not permit curve fitting using WinNonlin.

Mean plasma epinephrine concentrations obtained at the various times after injection or inhalation of epinephrine were compared using analysis of variance (ANOVA) and analysis of covariance (ANCOVA). The pharmacokinetic parameters were compared using the Tukey and Bonferroni multiple-range tests. PC-SAS computer programs were used. Differences were considered to be significant at $p \leq 0.05$.

Results

Mean baseline plasma epinephrine concentrations did not differ significantly before administration of epinephrine by the intramuscular, subcutaneous or intravenous routes, or before administration of the saline control injection. The mean plasma epinephrine concentration before administration of epinephrine by inhalation was significantly higher than the mean concentration before intravenous epinephrine injection ($p \leq 0.05$) (Table 1). In the control study, the C_{max} of endogenous epinephrine was 518 ± 142 pg/mL after the intramuscular injection of saline. Using the HPLC-EC analytical method, the epinephrine produced endogenously in the adrenal medulla cannot be distinguished from exogenously administered epinephrine. In the absence of a saline control for each of the study groups, the assumption was made that the endogenous epinephrine plasma concentrations obtained

Table 1. Rate and extent of epinephrine absorption after different routes of administration

Mean ± S.E.M.	Intramuscular	Subcutaneous	Inhalation	Intravenous	Saline control (0.1 mL)
Epinephrine dose (mg)	0.03	0.03	2.5	0.05	—
$C_{baseline}$ (pg/mL)	324 ± 145	530 ± 205	565 ± 154*	195 ± 89	460 ± 151
C_{max} (pg/mL)	7719 ± 3943	2692 ± 863	1196 ± 369	3544 ± 422[a]	518 ± 142
T_{max} (min)	32.5 ± 6.6**	111.7 ± 30.8	45.8 ± 19.2	—	—
$AUC_{0-3\ h}$ (ng/mL/min)	497 ± 120	211 ± 32	67 ± 18	236 ± 37	45 ± 15

* $p \leq 0.05$ intravenous.
** $p \leq 0.05$ subcutaneous
[a] Plasma epinephrine concentration shown was obtained at the first sample time, 5 min after epinephrine injection.
C_{max}: maximum plasma concentration (mean ± S.E.M. of individual C_{max} values from each subject, regardless of the time at which C_{max} was achieved). T_{max}: time at which maximum plasma epinephrine concentration was achieved (mean ± S.E.M. of individual T_{max} values from each subject). AUC: area under the plasma concentration versus time curve ($AUC_{0-\infty}$, intramuscular).

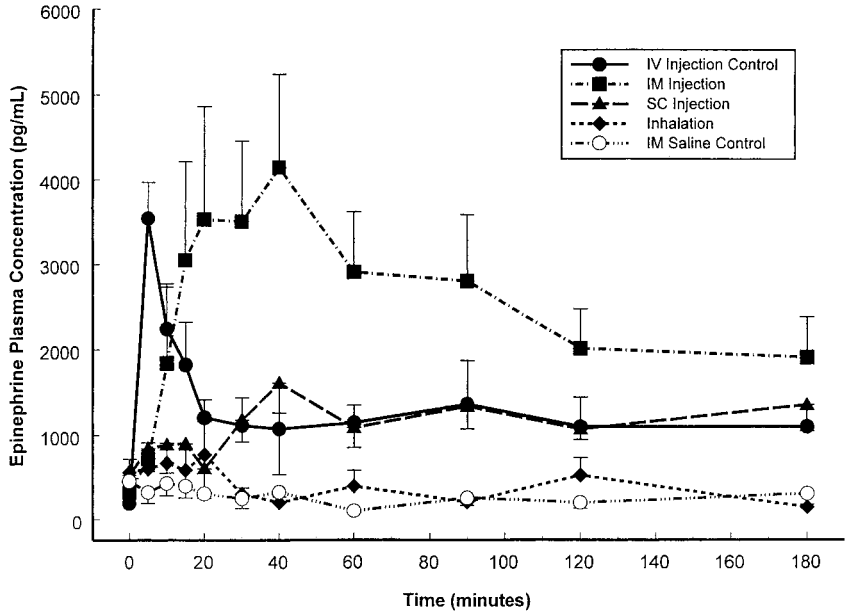

Figure 1. The epinephrine concentrations (pg/mL) versus time (min) are shown after epinephrine administration by intramuscular or subcutaneous injection, or by inhalation. Epinephrine concentrations on the control study days after intravenous injection of epinephrine and after intramuscular injection of normal saline are also shown; after saline injection, endogenous epinephrine concentrations were measured

after the intramuscular saline injection would be representative of the endogenous epinephrine plasma concentrations occurring after the administration of epinephrine by all other routes.

After the intramuscular injection of epinephrine, the maximum epinephrine concentration (C_{max}) was 7719 ± 3943 (mean $\pm$ S.E.M.) pg/mL, achieved at a time (T_{max}) of 32.5 ± 6.6 min (Figure 1). The variation in T_{max} after intramuscular injection did not permit calculation of the epinephrine terminal elimination half-life ($t_{1/2}$) after this route of administration. After subcutaneous injection, the C_{max} was 2692 ± 863 pg/mL, achieved at a T_{max} of 111.7 ± 30.8 min, which is significantly longer than the T_{max} after intramuscular injection ($p \leq 0.05$), although the C_{max} values were not significantly different. After inhalation, the C_{max} was 1196 ± 369 pg/mL, achieved at a T_{max} of 45.8 ± 19.2 min. Intravenous injection of epinephrine resulted in an epinephrine concentration of 3544 ± 422 pg/mL at 5 min, with a rapid terminal elimination $t_{1/2}$ of 11.0 ± 2.5 min.

The areas under the plasma epinephrine concentration versus time curves (AUCs) did not differ significantly after the different routes of injection and were 497 ± 120 ng/mL/min after intramuscular injection, 211 ± 32 ng/mL/min after subcutaneous injection, and 67 ± 18 ng/mL/min after inhalation, compared with 236 ± 37 ng/mL/min after intravenous injection and 45 ± 15 ng/mL/min after intramuscular saline injection. The AUC after intramuscular epinephrine injection was higher than that obtained after intravenous epinephrine injection, despite the larger dose administered intravenously. Absorption of the epinephrine from the site of the intramuscular injection, although slightly delayed, appeared to be complete.

Discussion

Failure to administer epinephrine promptly has been identified as the most important factor contributing to death in patients with systemic anaphylaxis [1–3], in which it has a variety of important pharmacological actions. Its α-adrenergic effects result in increased peripheral vascular resistance, blood pressure and coronary artery perfusion, and decreased peripheral vasodilation, angioedema and urticaria. Its β_1-adrenergic effects include positive inotropic and chronotropic activity. Its β_2-adrenergic effects result in bronchodilation and downregulation of the immediate hypersensitivity reaction secondary to activation of membrane-bound adenylate cyclase, elevation of cyclic adenosine monophosphate, and decreased release of chemical mediators of inflammation from mast cells and basophils [20,21]. As inhibition of mediator release by epinephrine is concentration-dependent and bidirectional, the possibility exists that low concentrations of epinephrine may actually enhance mediator release. Thus, the importance of rapidly achieving high initial epinephrine concentrations cannot be ignored.

The various recommended routes of epinephrine administration include intravenous, intramuscular or subcutaneous injection, and inhalation from a metered-dose inhaler [4–16,22–24]. The recommendations are based largely on clinical experience,

as there is no published comprehensive, prospective, randomized, controlled study in which the rate and the extent of absorption and the pharmacokinetics and pharmacodynamics of epinephrine have been compared after administration by each of these routes.

An important observation emerging from retrospective reviews of patients dying from anaphylaxis outside of hospital is that even when patients have access to self-injectable epinephrine, they are reluctant to use it, presumably because of a fear of injections [1,2]. Inhalation of epinephrine from a metered-dose inhaler [7,22–24] is recommended by some physicians as a useful alternative in patients: who are afraid of injections; whose previous episodes of anaphylaxis have been mild; or whose previous episodes have consisted predominantly of respiratory rather than cutaneous or vascular symptoms. In previous studies in small numbers of healthy adults who have been carefully instructed in inhaler technique including breath-holding, high-dose (10–30 puffs) inhaled epinephrine has been described as being both *superior* [22] and *inferior* [23] to subcutaneously injected epinephrine, with regard to the prompt achievement and maintenance of high plasma epinephrine concentrations. There are no published prospective comparisons of inhaled epinephrine with epinephrine injected either intramuscularly or intravenously.

In the study reported here, the low plasma concentrations found after inhalation, despite the relatively high epinephrine dose administered to the rabbits (2–3 puffs/kg versus ≤ 0.5 puff/kg used in humans) [22–24] are a concern, and suggest that this route of administration requires further investigation before it can be recommended for use in humans. Intramuscular rather than subcutaneous injection of epinephrine may be the optimal comparator for inhaled epinephrine, as maximum plasma epinephrine concentrations were higher and occurred more rapidly when epinephrine was injected by the former route. This is concordant with a recent, prospective, randomized, blinded, parallel-group study in children with a history of anaphylaxis, in whom we reported prompt epinephrine absorption after intramuscular injection, and variable absorption with peak plasma concentrations being reached as late as 120 min after subcutaneous injection [25]. Indeed, subcutaneously injected epinephrine is commonly used because of its vasoconstrictor effect, to delay the systemic absorption of co-administered medications such as local anaesthetics and to maintain them in high concentrations at or near the injection site.

This study in an animal model provides the foundation for prospective, comprehensive studies of epinephrine absorption and pharmacokinetics in humans at risk for anaphylaxis. It suggests that, in future studies, the intramuscular injection of epinephrine should be used as the 'gold standard' with regard to the prompt achievement of peak plasma epinephrine concentrations.

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