

Adrenaline (epinephrine) microcrystal sublingual tablet formulation: enhanced absorption in a preclinical model

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Abstract

Objectives For anaphylaxis treatment in community settings, adrenaline (epinephrine) administration using an auto-injector in the thigh is universally recommended. Despite this, many people at risk of anaphylaxis in community settings do not carry their prescribed auto-injectors consistently and hesitate to use them when anaphylaxis occurs. The objective of this research was to study the effect of a substantial reduction in adrenaline (Epi) particle size to a few micrometres (Epi microcrystals (Epi-MC)) on enhancing adrenaline dissolution and increasing the rate and extent of sublingual absorption from a previously developed rapidly disintegrating sublingual tablet (RDST) formulation in a validated preclinical model. **Methods** The in-vivo absorption of Epi-MC 20 mg RDSTs and Epi 40 mg RDSTs was evaluated in rabbits. Epi 0.3 mg intramuscular (IM) injection in the thigh and placebo RDSTs were used as positive and negative controls, respectively.

Key findings Epi_{mean}(standard deviation) area under the plasma concentration vs time curves up to 60 min and C_{max} from Epi-MC 20 mg and Epi 40 mg RDSTs did not differ significantly ($P > 0.05$) from Epi 0.3 mg IM injection. After adrenaline, regardless of route of administration, pharmacokinetic parameters were significantly higher ($P < 0.05$) than after placebo RDSTs administration (reflecting endogenous adrenaline levels).

Conclusion Epi-MC RDSTs facilitated a twofold increase in Epi absorption and a 50% reduction in the sublingual dose. This novel sublingual tablet formulation is potentially useful for the first-aid treatment of anaphylaxis in community settings.

Introduction

Administration of adrenaline (epinephrine) through an auto-injector in the mid-outer thigh is universally recommended in guidelines for treatment of anaphylaxis in community settings.^[1,2] The pharmacological effects of adrenaline are time dependent and concentration dependent.^[3] Ideally, as soon as anaphylaxis symptoms and signs are recognized, high adrenaline concentrations are required rapidly in the systemic circulation and in target organs to decrease release of inflammation mediators and prevent or reverse multi-organ system symptoms and signs, including upper and lower airway obstruction, respiratory distress, hypotension and shock.^[4] For decades, the EpiPen (Mylan, Canonsburg, PA, USA) and other adrenaline auto-injectors

have been recommended for this purpose. EpiPen contain a fixed adrenaline dose of either 0.15 mg or 0.3 mg. The adrenaline dose in the auto-injectors has a shelf life of 12 months.^[4]

To date, product development strategies by the pharmaceutical industry have addressed issues related to adrenaline administration by focusing only on injectable adrenaline formulations, for example, Auvi-Q (Sanofi Aventis U.S., Bridgewater, NJ, USA), an auto-injector with audio and visual electronic prompts and needle protection before and after use.^[5]

Reluctance of many patients to carry auto-injectors consistently and utilize them promptly when an anaphylactic

episode occurs are long-standing concerns; even patients with severe life-threatening anaphylaxis symptoms report failing to inject adrenaline in a timely manner.^[6,7] Use-related hazards such as unintentional injection into a digit or hand rather than into the thigh have been reported.^[8] In addition, parents have expressed fear of using an auto-injector incorrectly and of hurting their children when it is used to inject adrenaline.^[9]

Although orally inhaled adrenaline from a handheld metered-dose inhaler (or other device) is painless and potentially relieves mouth and throat swelling, most patients are unable to inhale a sufficient amount (10–20 inhalations for children; 20–30 inhalations for adults) quickly enough to increase their plasma adrenaline concentrations significantly and relieve their symptoms. Many patients do not like the bitter taste of the adrenaline. Some experience cough or dizziness during the inhalations.^[10]

We are developing a novel dosage formulation of adrenaline, a rapidly disintegrating sublingual tablet (RDST), for potential use in treatment of anaphylaxis in community settings.^[11,12] These readily portable, user-friendly RDST have the potential to provide optimal adrenaline dosing over a wide dose range; moreover, in this solid dosage formulation, adrenaline is stable for at least 7 years.^[13]

The non-invasive sublingual route offers a promising alternative for adrenaline administration. The high vascularity of the sublingual mucosa and the low molecular weight of adrenaline facilitate rapid absorption directly into the venous circulation through the sublingual veins.^[11,12,14–17] The RDSTs are hard enough to withstand shipping and handling, yet disintegrate rapidly *in vitro* to release adrenaline in <30 s.^[18–21] In previous preclinical studies, adrenaline 40 mg administered sublingually achieved plasma adrenaline concentrations similar to those reached after injection of adrenaline 0.3 mg in the thigh.^[11,12,14] Relatively high adrenaline doses are needed when the sublingual route of administration is utilized in order to create the concentration gradient required and facilitate absorption.^[11]

The rate and extent of drug dissolution and absorption can be enhanced by reducing the particle size into the micro-range or nano-range. Drug microcrystals and nanocrystals with few excipients can be fabricated, leading to production of virtually 100% pure drug.^[22]

We hypothesized that reduction in adrenaline particle size would significantly increase adrenaline dissolution and absorption, and permit reduction of the sublingual dose required to achieve adrenaline plasma concentrations equivalent to those after adrenaline 0.3 mg IM injection.

The objective of this study was to evaluate the effect of reduction in adrenaline particle size in the RDSTs on adrenaline sublingual absorption in a validated preclinical rabbit model. Here, for the first time, we explored the feasibility of reducing the sublingual adrenaline dose that we

previously determined would achieve the onset and duration of plasma adrenaline concentrations similar to those achieved after IM injection of 0.3 mg adrenaline.^[11,12,14]

Materials and Methods

Materials

(–)-Adrenaline (+) bitartrate (EpiBit) and 3,4-dihydroxybenzylamine, as internal standard for EpiBit HPLC analysis, were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ceolus PH-301 (microcrystalline cellulose), particle size 50 µm, was supplied by Asahi Kasei Chemicals Corp (Tokyo, Japan). Low-substituted hydroxypropyl cellulose-LH11, particle size 50 µm, was supplied by Shin-Etsu Chemical Co (Tokyo, Japan). Magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ, USA). Isopropyl alcohol, 99.5%, was purchased from BDH (VWR, West Chester, PA, USA).

Preparation of adrenaline bitartrate microcrystals

EpiBit microcrystals (EpiBit-MC) were prepared by a top-bottom technique using an LV-1 High Shear Fluid Processor ‘Microfluidizer’ (Microfluidics, Newton, MA, USA) equipped with a G10Z-type reaction chamber. Briefly, EpiBit (2.8 mg/ml), with no additional excipients, was suspended in 6 ml isopropyl alcohol, sonicated for 30 s and injected into the Microfluidizer. The suspension was processed at 30 000 Psi for one pass. The Microfluidizer receiving coil was immersed in ice to reduce the heat produced during the process. The resulting suspension was centrifuged at 27 200 g at 15°C for 30 min using an Avanti J-25 centrifuge (Beckman Coulter, Inc, Miami, FL, USA). The clear solvent was removed by aspiration, and the remaining particles were dried using CentriVap Concentrator (LabConco, Kansas City, MO, USA) at room temperature.

Particle size measurement

Mean particle size distribution (by volume) of raw EpiBit, before processing ($n=6$), was measured by laser diffraction using a Mastersizer (Malvern Instruments Inc., Westborough, MA, USA). Mean (Z-average) particle size distribution (by volume) of EpiBit-MC, after processing ($n=3$), was measured by light scattering using a ZetasizerZS90 (Malvern Instruments Inc.).

Manufacturing and quality control of tablets

RDSTs were formulated using the excipients and methods described in our previous studies.^[20,21] Three RDSTs batches containing no adrenaline (placebo RDSTs),

EpiBit-MC equivalent to Epi 20 mg, and EpiBit equivalent to Epi 40 mg were manufactured by direct compression. These RDSTs contained no lactose or bisulfite and were quality control tested for tablet weight variation, content uniformity and friability using the harmonized United States Pharmacopoeia (USP) standards and criteria.^[23,24] Tablet disintegration time was measured using a previously developed in-vitro disintegration test to simulate the sublingual environment.^[18–21]

Adrenaline content was analysed using a reverse-phase HPLC system with ultraviolet detection (Waters Corp., Milford, MA, USA) according to the USP method for Epi injections.^[25]

In-vivo studies

The research was conducted in a validated preclinical model in rabbits, an adrenaline-tolerant species. Current guidelines published by the Canadian Council on Animal Care were followed.^[26] The study was approved by the University of Manitoba Protocol Management and Review Committee on 6 March 2013, and the approval reference number is 11-065/1.

Using a prospective, placebo-controlled, randomized, four-way crossover study design, five New Zealand female white rabbits (mean $\pm$ standard deviation (SD) weight 3.6 ± 0.1 kg) were investigated on different study days at least 4 weeks apart, according to our previously published protocol.^[11,12,14] Each rabbit received Epi-MC 20 mg RDSTs, Epi 40 mg RDSTs, Epi 0.3 mg IM injection in the thigh from an EpiPen (positive control) and placebo RDSTs (negative control).

For sublingual tablet administration, the rabbit's mouth was gently opened using an oral speculum, and the tablet was placed underneath the tongue using flat forceps, followed by administration of 0.1–0.2 ml water to facilitate tablet disintegration. The rabbit's tongue was gently held over the tablet for 2 min to keep the tablet in the sublingual cavity, after which the mouth was rinsed with 30–40 ml of water to remove insoluble tablet residue.

For IM injection into the thigh, Epi 0.3 mg was administered from an EpiPen. The solution remaining in the EpiPen was then evacuated and frozen at -20°C for Epi content analysis according to the USP method.^[25]

Measurement of plasma adrenaline concentrations

An indwelling catheter (22G 1', BD, Mississauga, ON, Canada) was inserted into an ear artery at least 30 min before dose administration. Blood samples (2 ml) were obtained immediately before dosing (for measurement of baseline endogenous Epi) and at 5, 10, 15, 20, 30, 40 and 60 min after dosing.

Blood samples were transferred into Vacutainer plasma separation tubes containing EDTA (BD), refrigerated within 1 h of sampling and centrifuged at 1600g, 4°C . Plasma was stored at -20°C until analysis, then thawed at room temperature. Epi was extracted from plasma by solid-liquid extraction process with an efficiency of 78–83%. Epi concentrations were measured using an HPLC system composed of a 2690 Alliance separations module and a 2465 electrochemical detector (Waters Corp.) using a reversed-phase Nova-Pak C18, 3.9×150 mm, column and 3,4-dihydroxybenzylamine as internal standard.^[27–29] A glassy carbon working electrode was set at +600 mV, an in-situ silver/silver chloride (ISAAC) reference electrode was used and the detector sensitivity was set at 10 nA. High-range and low-range calibration curves were prepared. The low-range curve, 0.1–1.0 ng/ml, was linear with a coefficient of variation of 0.4% at 0.1 ng/ml and 0.1% at 1.0 ng/ml. The high-range curve, 1.0–10.0 ng/ml, was linear with a coefficient of variation of 0.1% at 1.0 ng/ml and 0.1% at 10.0 ng/ml.

Data analysis

The maximum plasma Epi concentration (C_{max}), the time at which C_{max} was achieved (T_{max}), and the area under the plasma concentration vs time curves up to 60 min ($\text{AUC}_{0-60 \text{ min}}$) were calculated from the plasma Epi concentration vs time plots of each individual rabbit using a non-compartmental model by WinNonlin 5.3 (Pharsight, Mountain View, CA, USA). The $\text{AUC}_{0-60 \text{ min}}$, C_{max} and T_{max} values for each rabbit were compared using analysis of covariance and Tukey–Kramer tests at a significance level of $P < 0.05$ using NCSS Statistical Analysis Software (NCSS, Kaysville, UT, USA).

Results

Particle size measurement

Mean (SD) particle size distribution of the raw EpiBit crystals was 131.8 ± 10.5 μm . The particle size distribution ranged from 39.8 ± 3.0 μm (10th percentile) to 254.8 ± 20.1 μm (90th percentile) and the median (50th percentile) was 113.6 ± 9.1 μm .

After processing EpiBit-MC using the Microfluidizer, mean (SD) particle size distribution was 2.5 ± 0.4 μm with a polydispersity of 0.185 ± 0.019 .

In-vivo studies

All RDSTs disintegrated in less than 30 s and met the harmonized USP standards for tablet weight variation, content uniformity and friability.

Mean (SD) of Epi dose injected using EpiPen auto-injectors was 0.29 ± 0.02 mg as calculated by multiplying the Epi concentration, measured in the solution remaining

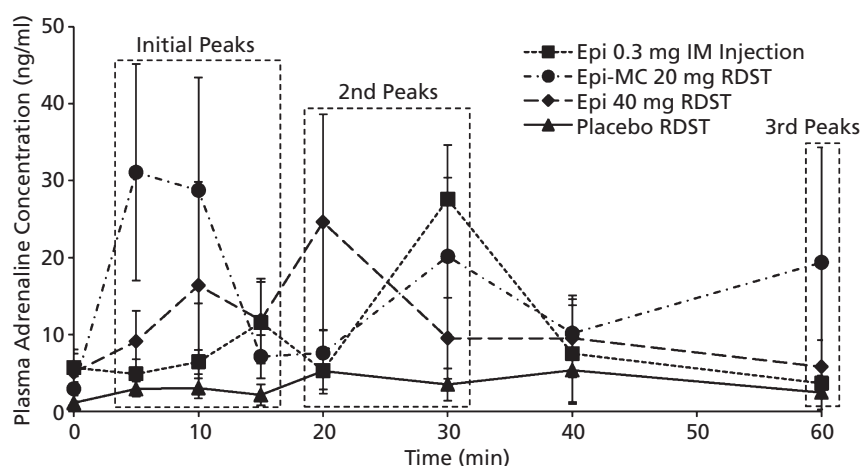


Figure 1 Plasma adrenaline concentration vs time plots after sublingual administration of adrenaline (Epi), Epi microcrystals (Epi-MC) or placebo rapidly disintegrating sublingual tablet (RDSTs) and after Epi IM injection. Mean (standard deviation (SD)) area under the plasma concentration vs time curve (AUC), C_{max} and T_{max} after administration of Epi-MC 20 mg RDSTs, Epi 40 mg RDSTs and Epi IM injections were not significantly different ($P > 0.05$, $n = 5$).

Table 1 Adrenaline absorption after sublingual administration of placebo, adrenaline (Epi) and Epi microcrystal (Epi-MC) tablets and Epi IM injection in the thigh

Mean (SD) ^a	Sublingual tablets			IM injection
	Epi-MC 20 mg	Epi 40 mg	Placebo	EpiPen®
Adrenaline dose (mg)	20.0	40.0	0	0.3
AUC (ng/ml/min)	942.0 ± 243.7*	678.0 ± 149.0*	220.1 ± 78.0	592.0 ± 122.3*
AUMC (ng/ml/min ²)	27 590.8 ± 11 627.0*	17 616.6 ± 4953.1*	6835.7 ± 3036.7	17 042.5 ± 4944.4*
$C_{baseline}$ (ng/ml)	2.9 ± 1.6	5.0 ± 3.0	1.1 ± 1.2	5.6 ± 1.9**
C_{max} (ng/ml)	38.0 ± 9.9*	31.7 ± 10.1*	7.5 ± 3.0	27.6 ± 7.0*
MRT (min)	28.8 ± 6.9	25.8 ± 2.9	30.2 ± 5.2	28.4 ± 2.5
T_{max} (min)	28.0 ± 29.3	20.0 ± 7.1	33.3 ± 17.5	30.0 ± 0.0

AUC, area under the plasma concentration vs time curve; AUMC, area under the first moment curve; $C_{baseline}$, baseline plasma concentration (endogenous adrenaline); C_{max} , maximum plasma concentration (mean ± standard deviation (SD) of individual C_{max} values from each rabbit, regardless of the time at which C_{max} was achieved); MRT, mean residence time; T_{max} , time at which maximum plasma adrenaline concentration was achieved (mean ± SD of individual T_{max} values from each rabbit). ^a $n = 5$. * $P < 0.05$ from placebo tablet but not from each other. ** $P < 0.05$ from placebo tablet but not from others.

in each EpiPen after injection, by the stated injected volume (0.3 ml).

Mean (SD) plasma Epi concentration vs time plots after the sublingual administration of Epi-MC 20 mg RDSTs, Epi 40 mg RDSTs and placebo RDSTs, and the IM injection of Epi 0.3 mg using EpiPen are shown in Figure 1. Mean (SD) $AUC_{0-60 \text{ min}}$, AUMC, $C_{baseline}$ (endogenous Epi), C_{max} , MRT and T_{max} values are shown in Table 1. No adverse effects were observed.

Mean (SD) AUC after the administration of Epi-MC 20 mg RDSTs (942.0 ± 243.7 ng/ml/min), Epi 40 mg RDSTs (678.0 ± 149.0 ng/ml/min) and Epi 0.3 mg IM (592.0 ± 122.3 ng/ml/min) did not differ significantly from each other; however, they were significantly higher than the values found after placebo RDSTs administration (220.1 ± 78.0 ng/ml/min) that reflected endogenous adrenaline.

Mean (SD) C_{max} values after the administration of Epi-MC 20 mg RDSTs (38.0 ± 9.9 ng/ml), Epi 40 mg RDSTs (31.7 ± 10.1 ng/ml) and Epi 0.3 mg IM (27.6 ± 7.0 ng/ml) did not differ significantly from each other; however, they were significantly higher than after placebo RDSTs administration (7.5 ± 3.0 ng/ml).

Mean (SD) T_{max} after Epi-MC 20 mg RDSTs (28.0 ± 29.3 min), Epi 40 mg RDSTs (20.0 ± 7.1 min), Epi 0.3 mg IM (30.0 ± 0.0 min) and placebo RDSTs (33.3 ± 17.5 min) did not differ significantly.

Discussion

Anaphylaxis guidelines recommend that patients at risk of anaphylaxis episodes in community settings learn to recognize anaphylaxis symptoms and signs promptly and

administer adrenaline from an auto-injector as soon as anaphylaxis is recognized.^[1,2] Although there has been progress in adrenaline auto-injector development in the past few years, for example, with the introduction of the innovative Auvi-Q,^[5] some limitations of this dosage form remain. In addition to those described previously,^[6–9] these include availability of only three fixed doses (adrenaline 0.15, 0.3 and, in some countries, 0.5 mg) rather than a range of doses and the limited shelf life of adrenaline in a liquid formulation necessitating annual replacement of unused auto-injectors.^[4]

RDSTs have been developed to improve the likelihood of prompt adrenaline administration in anaphylaxis in community settings. In a proof-of-concept preclinical study, first-generation sublingual adrenaline tablets resulted in plasma adrenaline concentrations that were similar to those measured after intramuscular injection of 0.3 mg adrenaline from EpiPens.^[11,12] Subsequently, second-generation adrenaline sublingual tablets in which adrenaline dissolution time was optimized^[19] and the intrinsic bitter taste of adrenaline was successfully masked^[30] were developed. Studies of these tablets in this preclinical model confirmed that adrenaline was absorbed as rapidly from the second-generation tablets as from IM adrenaline injections.^[14]

In the study reported here, we enhanced the sublingual absorption of adrenaline by reducing the particle size of EpiBit crystals. This reduction in particle size results in increased saturation solubility and rate of dissolution, which increases the concentration gradient across the sublingual mucosa and enhances the rate and extent of absorption.^[31] This is particularly important for sublingual drug delivery because of the small volume of saliva available for drug dissolution and the short sublingual residence time.

In the preclinical model, we report here for the first time that EpiBit-MC significantly increased adrenaline sublingual absorption compared to raw EpiBit crystals. We determined that RDSTs containing an adrenaline dose of 20 mg as EpiBit-MC resulted in AUC, $C_{\max}$ and $T_{\max}$ values similar to those achieved following Epi IM injection into the thigh from an EpiPen (Epi_{mean} dose 0.29 mg) and Epi 40 mg RDSTs using raw EpiBit crystals. The reduction in the particle size of EpiBit increased adrenaline absorptivity twofold.

As in all our previous adrenaline bioavailability studies, regardless of adrenaline dose or route of administration,^[11,12,14] the adrenaline vs concentration time curve was characterized by multiple intermittent adrenaline peaks. The initial peak was likely due to rapid adrenaline

absorption, followed by vasoconstriction at the site of administration in the sublingual mucosa, leading to temporary reduction in absorption, accumulation at the site, then followed by vasodilation and a second phase of rapid absorption, leading to a second and higher peak. By using the EpiBit-MC, we achieved higher initial first and second peaks within the first 10 min, followed by later peaks (Figure 1), likely resulting from increased saturation solubility and improved dissolution rates of EpiBit microcrystals compared to the larger EpiBit crystals. This led to an even higher concentration gradient across the sublingual membrane, which, despite the reduced dose of the EpiBit-MC, resulted in absorption of a larger amount of adrenaline as reflected in the magnitude of the peaks before the adrenaline produced its initial local vasoconstriction.

We conclude that reducing the particle size of EpiBit to approach the nanoparticle-size range improved its in-vivo absorption from RDSTs by twofold and facilitated reduction of the sublingual adrenaline dose by 50%.

This bioavailability study needs to be repeated in a phase 1 trial in humans. Also, studies of adrenaline absorption from the sublingual mucosa should be performed in animal models of anaphylaxis because swelling of the oral and sublingual mucosa might affect the rate and extent of adrenaline absorption.

Conclusion

We have demonstrated that reducing the particle size of EpiBit to a size approaching the nanoparticle range improved its in-vivo absorption from RDSTs by twofold. Epi-MC RDSTs reduced the required sublingual Epi dose by 50%. These tablets are suitable for phase I studies in humans and may have the potential for the first-aid emergency treatment of anaphylaxis in community settings.

Declarations

Conflict of interest

Mutasem M. Rawas-Qalaji, Ousama Rachid, F. Estelle R. Simons and Keith J. Simons have pending patent applications. Belacryst A. Mendez and Annette Losada have no conflict of interest to declare.

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