FORMULATION AND ASSESSMENT OF FAST-DISINTEGRATING SUBLINGUAL EPINEPHRINE TABLETS FOR THE POTENTIAL EMERGENCY TREATMENT OF ANAPHYLAXIS

By

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Formulation and Assessment of Fast-Disintegrating Sublingual Epinephrine Tablets for the Potential Emergency Treatment of Anaphylaxis

By

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirement of the degree

Of

Doctor of Philosophy

DEDICATION

To my father Mohamad Rawas-Qalaji and my mother Wedad Duqsi (in memoriam) I would like to dedicate this work.

ABSTRACT

Objectives: To formulate, characterize, and evaluate the stability of sublingual (SL) epinephrine (E) tablets that are bioequivalent to E 0.3 mg intramuscular injection (IM) for the feasibility of SL E administration for the potential out-of-hospital emergency treatment of anaphylaxis. ***Methods:*** E stability was tested at 37 °C in buffer, pH range 5.8-7.1, and human saliva. The effect of changing tablet dimensions on fast-disintegrating tablets (FDT) characteristics was evaluated in two FDT formulations, 0% and 10% epinephrine bitartrate (EB), prepared by direct compression, using a range of forces and different die sizes and punch shapes. Also, four FDT formulations, 0%, 6%, 12%, and 24% EB equivalent to E 0, 5, 10, and 20 mg respectively, prepared and compressed at a range of forces, were used to evaluate the effect of increasing EB load on FDT characteristics. Tablet weight variation (WV), content uniformity (CU), friability (F), thickness, hardness (H), disintegration time (DT), and wetting time (WT) were measured. Three FDT batches, E 10 mg, 20 mg, and 40 mg, were stored in tightly closed, opaque, plastic containers with desiccants at 25 °C, 5 °C, and 5 °C under nitrogen (5 °C-N_2) to evaluate the stability of FDT. In a 5-way crossover study, SL FDT containing E 0, 10, 20, and 40 mg were compared with E 0.3 mg IM in the thigh in a validated rabbit model to determine the SL E dose required. Also, three additional distinct FDT formulations all containing E 40 mg were prepared to evaluate the effect of changing excipients on the relative bioavailability of E. E was analyzed using HPLC with UV or EC detectors.

Results: E doses at baseline and at all sample times up to 20 min (n=5) in buffer pH 5.8, buffer pH 7.1, and buffer pH 5.8 with effervescent excipients, and in saliva were not significantly different. The 8/32", 10/32", and 11/32" dies resulted in FDT thickness ranges of 0.25"-0.19", 0.17"-0.1", and 0.16"-0.08", respectively. For 10% EB FDT, the DT and WT (n=6) were ≤10 sec and ≤30 sec, respectively, at H <4 kg. No difference in DT and WT was observed between concave and flat tablets. FDT loaded with 0, 6, 12, and 24% EB were within the USP limits for WV and CU. At H (±SD) ≥2.3 ± 0.2 kg (n=6), all FDT passed the USP F test. At H ≤3.1 ± 0.2 kg, all FDT resulted in DT and WT <10 sec and <30 sec, respectively. E doses (n=6) in E 10 mg and in E 20 mg FDT stored for 12 months, and in E 40 mg FDT stored for 20 months at 25 °C, 5 °C, and 5 °C-N_2 did not differ significantly from controls and from each other. Using this FDT formulation, the area under the curve (AUC), maximum concentration (C_{max}), and time at which C_{max} was achieved (T_{max}) did not differ significantly after the administration of SL E 40 mg and E 0.3 mg IM (n=5). This E 40 mg FDT formulation and the additional 3 distinct E 40 mg FDT formulations resulted in similar H, DT, and WT (n=6). However, AUC obtained after the administration of the 3 distinct SL FDT formulations were significantly lower than after E 0.3 mg IM (n=5). ***Conclusions:*** E was stable in saliva and in this tablet formulation and was absorbed after SL administration. The selection of excipients and H resulted in an E 40 mg SL FDT that was bioequivalent to E 0.3 mg IM. This novel SL FDT E formulation warrants further development as a feasible alternative for the first-aid emergency treatment of anaphylaxis in humans.

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GLOSSARY

5 °C-N_2:	5 °C under nitrogen
AUC:	area under the plasma epinephrine concentration versus time curve
$C_{basline}$:	baseline plasma concentration (endogenous epinephrine)
C_{max}:	maximum plasma epinephrine concentration
CF:	compression force
CP:	concave punches
CU:	content uniformity
CV%:	coefficient of variation (%)
DHBA:	dihydroxybenzylamine
DT:	disintegration time
E:	epinephrine
EB:	epinephrine bitartrate
EC:	electrochemical detection
EDR:	epinephrine dose remaining
EDTA:	ethylenediaminetetraacetic acid
EE:	effervescent excipients
EPCs:	epinephrine plasma concentrations
F:	friability
FDT:	fast-disintegrating tablet
FP:	flat punches
H:	hardness
HPLC:	high performance liquid chromatography

IM:	intramuscular injection
LH:	low-substituted hydroxypropyl cellulose (three hydroxyl groups are etherified with propylene oxide)
R^2:	correlation of coefficient
SD:	standard deviation
SEM:	standard error of the mean
SL:	sublingual
T:	thickness
T_{max}:	time of maximum plasma epinephrine concentration
USP:	*United States Pharmacopeia*
UV:	ultraviolet detection
WT:	wetting time
WV:	weight variation

CHAPTER I: Introduction

1.1. Research Rational

There is universal agreement that prompt epinephrine injection is the drug of choice for the treatment of anaphylaxis (Lieberman, 2003; McLean-Tooke *et al.*, 2003; Sampson *et al.*, 2006; Simons, 2004). The recommended epinephrine dose for the treatment of anaphylaxis is 0.3-0.5 mg in adults and 0.01 mg/kg, up to a maximum of 0.3 mg, in children, given by intramuscular injection (Lieberman, 2003; McLean-Tooke et al., 2003; Sampson et al., 2006; Simons, 2004). These recommendations are based on clinical experience and/or studies in healthy volunteers (Simons *et al.*, 2001b), rather than on prospective, randomized, double-blind, placebo-controlled dose-ranging studies in patients experiencing anaphylaxis, which are impossible to perform from the ethical standpoint (Simons, 2004).

Most anaphylactic reactions occur unexpectedly in the community due to foods, insect stings, medications, natural rubber latex, and other triggers (Lieberman, 2003; Sampson et al., 2006; Simons, 2004). For out-of-hospital emergency treatment of anaphylaxis, epinephrine auto-injectors such as EpiPen®, EpiPen Jr® (Dey LP, Nappa, CA), Twinject 0.3 mg®, and Twinject 0.15 mg® (Verus Pharmaceuticals, Inc. San Diego, CA) are prescribed, however, self-injectable epinephrine is underutilized when anaphylaxis occurs (Bock *et al.*, 2001; Gold & Sainsbury, 2000). The drawbacks of epinephrine auto-injectors

include: high cost which limits affordability and availability worldwide (Simons, 2005); perceived large size and bulkiness; limitations on repeat dosing (if required) (Korenblat *et al.*, 1999); fear and anxiety associated with the use of needles (Simons, 2004); and dosing errors due to incorrect technique of administration (Gold & Sainsbury, 2000; Sicherer *et al.*, 2000). In addition, it is impossible to give an accurate dose to infants and to many young children using currently available auto-injectors, which provide only two different premeasured, fixed epinephrine doses, 0.15 mg and 0.3 mg (Simons, 2004). Alternatives to an epinephrine auto-injector, such as an epinephrine ampule/syringe/needle or as an epinephrine metered dose inhaler are impractical with regard to rapid and accurate dosing (Simons, 2004; Simons *et al.*, 2001a; Simons *et al.*, 2000).

The sublingual route of administration is a promising alternative route for epinephrine administration. Drugs that are absorbed sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Motwani *et al.*, 1991; Price *et al.*, 1997). Lipophilic drugs with a low molecular weight such as epinephrine are likely absorbed across the sublingual mucosa into the venous circulation by transcellular diffusion (Birudaraj *et al.*, 2005), a mechanism driven by the concentration gradient (Sherwood, 2004).

1.2. Research Hypothesis

It was hypothesized that epinephrine could be formulated in novel, fast-disintegrating tablets for sublingual administration, which, with the selection of an appropriate sublingual dose, would result in plasma epinephrine concentrations similar to those obtained following 0.3 mg epinephrine intramuscular injection. These sublingual tablets could have the potential as an alternative route of epinephrine administration for the emergency treatment of anaphylaxis.

1.3. Research Objectives

The overall objective was to formulate sublingual epinephrine tablets that would result in a similar rate and extent of absorption of epinephrine to that obtained following epinephrine 0.3 mg intramuscular injection. The objectives were selected to answer, in a systematic sequence, the most important questions anticipated during the progression of this research project.

These objectives were: 1) to evaluate the stability of epinephrine in human saliva; 2) to evaluate the effect of changing tablet dimensions on tablet characteristics when formulating sublingual fast-disintegrating epinephrine tablets; 3) to evaluate the effect of increasing epinephrine load on tablet characteristics for dose ranging studies 4) to determine the sublingual epinephrine dose required using these tablets that will result in plasma epinephrine concentrations similar to those obtained following epinephrine 0.3 mg intramuscular injection, using a validated rabbit model; 5) to evaluate the effect of changing excipients (non-medicinal ingredients) on the rate and extent

of epinephrine absorption from various sublingual tablet formulations. Tablet characteristics will always conform to those defined for the dose ranging sublingual tablet formulation. The various sublingual epinephrine tablet formulations will be compared with the epinephrine 0.3 mg intramuscular injection, using the same validated rabbit model.

1.4. Significance of Research and Overall Implications

The readily accessible, convenient, sublingual route of administration has long been used to administer medications requiring prompt onset of action, such as nitroglycerine. The high vascularity of the sublingual mucosa facilitates rapid drug absorption directly into the venous circulation through the sublingual and frenular veins, bypassing the gastrointestinal tract, the hepatic portal circulation, and hepatic first-pass metabolism (Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Motwani *et al.*, 1991; Price *et al.*, 1997).

Specially designed fast-disintegrating sublingual tablet formulations would expedite the availability of epinephrine for rapid absorption by the sublingual mucosa blood vessels (Ishikawa *et al.*, 2001). These tablets could be good candidates for out-of-hospital treatment of emergency conditions and for dugs that are extensively metabolized after oral administration (Bredenberg *et al.*, 2003; Saxena *et al.*, 2005). Drug absorption could be terminated if necessary by removing the tablet from the mouth. Sublingual epinephrine tablets could be formulated in a range of epinephrine doses to provide accurate doses for individuals over a wide range of body weights. Multiple doses would be readily

available. The tablets would be unobtrusive to carry and to self-administer. They should be less expensive to produce than the currently available auto-injectors are. Finally, they should increase the utilization of epinephrine for out-of-hospital emergency treatment of anaphylaxis.

1.5. Organization of Thesis

This thesis is organized into ten chapters as a sandwich thesis (manuscripts within a thesis). The entire thesis has been assigned sequential page numbers and has consistent format and font (Faculty of Graduate Studies, 2005).

The first chapter "Introduction" includes the Research Rational, Hypothesises, Objectives, and Significance and Overall Implications, and this section "Organization of Thesis".

The second chapter "Literature Review" includes four sections; Anaphylaxis, Epinephrine, Limitations of the Available Epinephrine Route of Administration, and Sublingual Route as Alternative Route of Administration for Epinephrine.

The third chapter is "HPLC Analysis of Epinephrine", which is required for epinephrine quantification. It is organized into four sections; Introduction, Materials and Methods, Results and Discussion, References, and Tables and Figures.

The fourth to the ninth chapters include the research studies conducted to pursue the objectives and to prove the hypothesis of this research. Each chapter, from the third to the ninth, stands alone and addresses one of the main objectives, stated in "Research Objectives" section (1.3), in a systematic and

sequential manner. Each chapter is organized in a manuscript format, which contains its own Abstract, Introduction, Materials and Methods, Results and Discussion, Conclusions, References, and Tables and Figures. Each chapter represents an original manuscript in a peer-reviewed journal. These papers are either published, accepted for publication, or ready for submission. Written permission from the coauthors and the copyright holder of each published paper included in this thesis has been obtained and attached to the first two pages of the related chapter.

The tenth and final chapter "Conclusions" includes the overall conclusions for all the research studies described in chapters four to nine.

The sandwich thesis format has been selected because most of my research studies have been published (chapters six, eight, and nine), accepted for publication (chapter five), or prepared for submission as a short communication, letter to the editor, or note (chapters four and seven).

My contribution to each manuscript in this thesis has been acknowledged, as I am the first author of all the manuscripts. All of the study designs were based on my evaluation of the literature and in discussion with my advisor and co-investigators. I have performed all of the research studies described in each chapter alone, or with the assistance of my advisor and/or co-investigators where necessary. All the figures, tables, and photos reported in the Results section of each chapter were designed, drawn, and captured by myself and reviewed and modified where necessary following discussion with my advisor and/or co-investigators. All the ideas, justifications and explanations, and the literature

review in the Discussion section of each chapter were my work alone, and/or with the assistance of my advisor and/or co-investigator when necessary. All the papers were written by myself and reviewed, corrected and modified by the coauthors if necessary.

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CHAPTER II: Literature Review

2.1. Anaphylaxis

The phenomenon of anaphylaxis although recognized sporadically for years was not understood until 1901, when Paul Portier and Charles Richet discovered the explanation while attempting to immunize and protect dogs with *Actinia* extracts. The dogs unexpectedly died after repeated injection of nonlethal doses. The word "anaphylaxis", which means the opposite of protection (phylaxis), was then coined by Charles Richet in 1902, and for his description of the disease he received the Nobel prize in Medicine or Physiology in 1913 (Richet, 1913; Ring *et al.*, 2004).

2.1.1. Definition of Anaphylaxis

Anaphylaxis has been perceived or known for a long time as a rare disease (Simons, 2006) that has no universal definition and diagnosis criteria (Sampson *et al.*, 2006). In an attempt to resolve this problem, the National Institute of Allergy and Infectious Disease (NIAID) and the Food Allergy and Anaphylaxis Network (FAAN) arranged two symposia in April 2004 and in July 2005, which included representatives from thirteen different organizations in North America and sixteen different organizations in North America, Europe, and Australia, respectively. During the second symposium that commenced in July 2005, the participants agreed that a brief and broad definition would be most

useful for the medical community and for the public and decided that the definition of anaphylaxis is "a serious allergic reaction that is rapid in onset and may cause death" (Sampson *et al.*, 2006).

2.1.2. Epidemiology and Etiology

Although anaphylaxis is a disease of modern times, the true incidence is unknown. Most anaphylactic reactions occur in the community rather than in a healthcare setting. Many individuals with mild or moderate symptoms do not report to emergency departments (Simons, 2006). The lack of a universal definition and precise diagnostic criteria for anaphylaxis has hindered researchers from conducting optimal prospective surveillance studies to determine the true prevalence of the disease (Sampson *et al.*, 2006).

Food is the most common cause of anaphylaxis, accounting for more than one third of adults and more than one half of children treated in emergency departments for anaphylaxis (Sampson, 2003). The annual occurrence rate of food-induced anaphylaxis is 1-9 per 100,000 people in the United States, England, France, Switzerland, Sweden, Australia, and Italy (Moneret-Vautrin *et al.*, 2005; Sampson, 2003). In the United States, it is estimated, based on a population of 280 million, that 30,000 food-induced anaphylactic episodes occur each year, resulting in 2,000 hospitalizations and 150 to 200 deaths (Sampson, 2003; The Food Allergy & Anaphylaxis Network (FAAN), 2006; World Allergy Organization (WAO), 2006). The most common food triggers are peanuts, the commonest cause in children (Lieberman, 2003), tree nuts (walnuts, hazelnuts,

cashews, pistachio nuts, brazil nuts, almonds), fish, shellfish (shrimp, lobster, scallops), milk (cow, goat), eggs, seeds (cotton seeds, sesame, psyllium, mustard), and fruits (kiwi) (Sampson, 2003; World Allergy Organization (WAO), 2006). Food sensitivity can be so severe that systemic allergic reactions can occur due to aerosolized particle inhalation, such as odors of cooked fish or peanuts.

Penicillin is considered the most common cause of drug-induced anaphylaxis with a frequency of 1-5 per 10,000 patient during penicillin therapy and 1 fatality per 50,000 to 100,000 patients (Lieberman, 2003; Moneret-Vautrin *et al.*, 2005). Muscle relaxants account for 80% of the allergic reactions occurring during general anesthesia with a rate of 1 per 4,500 and a mortality rate of 3.4-6% (Lieberman, 2003; World Allergy Organization (WAO), 2006). Aspirin and NSAIDs can also cause anaphylaxis (Lieberman, 2003; Moneret-Vautrin *et al.*, 2005). Sulfiting agents (sodium and potassium sulfites, bisulfites, metasulfites) added to food and drinks to prevent discoloration, and to variety of medications as preservatives also can cause allergic reactions in susceptible individuals (World Allergy Organization (WAO), 2006).

The incidence of allergic reactions to *Hymenoptera* stings ranges between 0.4 and 3% with 25-50 fatalities per year (Lieberman, 2003; World Allergy Organization (WAO), 2006).

Allergic reactions to latex are a recognized problem among health care workers. The percentage of allergic reactions due to latex ranges between 8 and 17% of exposed health care workers (Lieberman, 2003).

Two thirds of the adult patients with anaphylaxis who are referred to an allergist-immunologist are found to suffer from idiopathic anaphylaxis. In United States, the number of individuals with idiopathic anaphylaxis are estimated to be between 20,592 and 47,024 case (Lieberman, 2003).

2.1.3. Pathophysiology

Allergic reactions occur when an antigen (allergen) binds to antigen-specific IgE antibody affixed to the surface of previously sensitized mast cells and basophils. This leads to activation of mast cells and basophils and results in a subsequent release of preformed mediators, stored in granules, as well as the synthesis of new mediators (Figure 1). These allergic mediators, which include histamine, leukotrienes, prostaglandins, and tryptase, are responsible for causing the signs and symptoms of anaphylaxis (Ewan, 1998; Lieberman, 2003; Ring *et al.*, 2004). Rapid systemic release of large quantities of allergic mediators cause smooth muscle spasm, capillary leakage, and mucosal edema resulting in the signs and symptoms of anaphylaxis (Ewan, 1998; Lieberman, 2003).

Anaphylactoid reactions result in symptoms similar to anaphylaxis through direct action on mast cells, but without the involvement of IgE antibodies (Ewan, 1998; Lieberman, 2003; Ring et al., 2004).

2.1.4. Signs and Symptoms

The signs and symptoms of anaphylaxis may be isolated to one organ system such as the larynx or the blood vasculature, but more often involve

a number of systems (World Allergy Organization (WAO), 2006). The onset, sequence, and severity of the symptoms vary among patients and may even vary in the same patient during repeated episodes (Sampson, 2003). Symptoms usually begin within 5 to 30 min after the exposure of an allergic patient to an allergen. However, they can develop within seconds or be delayed for several hours. These symptoms may include cutaneous, respiratory, gastro-intestinal, and cardiovascular manifestations.

Cutaneous manifestations are the most common. Eighty-eight percent of patients experience these symptoms (Lieberman, 2003; Ring *et al.*, 2004). They may include flushing, urticaria, pruritus, and angioedema (Lieberman, 2003; Sampson, 2003).

Respiratory manifestations are the next most common in occurrence. Forty-seven percent of patients experience these symptoms (Lieberman, 2003), that may include airway obstruction due to angioedema, bronchospasm, chest tightness, cough, wheezing, rhinitis, sneezing, congestion, and rhinorrhea (Lieberman, 2003; Sampson, 2003; World Allergy Organization (WAO), 2006).

Gastro-Intestinal manifestations may include abdominal pain, nausea, vomiting, and diarrhea. Thirty percent of the patients experience these symptoms (Lieberman, 2003; Sampson, 2003).

Cardiovascular manifestations may include faintness, hypotension, hypovolemic shock, arrhythmias, syncope, and chest pain (Sampson, 2003; World Allergy Organization (WAO), 2006).

A late phase response, which is termed *biphasic anaphylaxis*, refers to the recrudescence of symptoms after an apparent temporary resolution, asymptomatic period. The onset may vary from 1 to 4 hours after the initial reaction (Lieberman, 2003; Sampson, 2003). Protracted anaphylaxis may also occur with symptoms persisting for day after a single allergen exposure. It is characterized by repeated recurrences interrupted by asymptomatic periods (Lieberman, 2003; World Allergy Organization (WAO), 2006).

2.1.5. Treatment

The aim of the initial therapy is to maintain effective respiratory and cardiovascular systems. There is a universal agreement that prompt intramuscular epinephrine injection is the drug of choice for the treatment of anaphylaxis regardless of the cause of anaphylaxis. The recommended dose is 0.01 mg/kg with a maximum dose of 0.5 mg administered intramuscularly, preferably in the thigh (Simons *et al.*, 2001b), every 5 to 15 minutes as necessary (Sampson *et al.*, 2006).

Other treatment measures can also be considered but as a second-line treatment of anaphylaxis. High-flow oxygen and inhaled β_2-agonists should be administered for patients experiencing respiratory symptoms. Aggressive fluid resuscitation and potent vasopressors, e.g. vasopressin, might be required to overcome vasodilation if epinephrine failed to maintain systolic blood pressure. Antihistamines (H_1- and H_2-antagonists) are useful for the treatment of cutaneous

manifestations. Corticosteroids might prevent protracted or biphasic reactions (Sampson et al., 2006).

2.2. Epinephrine

Epinephrine is the active, naturally occurring, principle hormone of the suprarenal (adrenal) gland in the medulla. It was isolated by Vulpian in 1856 (Dubin, 1925; Payne, 1961). The effect of epinephrine on blood pressure was first observed by Oliver and Schafer in 1894 (Payne, 1961). Epinephrine was initially obtained in its impure form by Abel and Crawford in 1897, and was later purified by Takamine in 1901. The chemical structure of epinephrine was established in 1903, and it was first synthesized by Stolz and Flaecher in 1904 (Dubin, 1925). Epinephrine was introduced into western medicine in 1924 (Hoffman & Lefkowitz, 1996).

2.2.1. Physiochemical Properties

Epinephrine, (-)-3,4-dihydroxy-α-[(methylamino)methyl] benzyl alcohol (Figure 2), is a white or light brownish, odourless, crystalline powder that gradually darkens on exposure to light and air. It is very slightly soluble in water (Keefe, 2000). It is available as very water-soluble and chemically stable hydrochloride and bitartrate salts (Sciarra *et al.*, 1972). Epinephrine bitartrate was used in this research because it was readily obtainable as the pure L-isomer, the pharmacologically active form.

Epinephrine bitartrate, (-)-3,4-dihydroxy-α-[(methylamino)methyl] benzyl alcohol (+)-tartrate (1:1) salt, is a white, greyish white, or light brownish grey, odourless, crystalline powder that slowly darkens on exposure to light and air (Keefe, 2000). It has poor tablet manufacturing properties, such as flowability and compressibility, as do most active medicinal ingredients.

In aqueous solutions, epinephrine bitartrate is very soluble, 1 gm in 3 mL of water (Keefe, 2000). It is unstable in neutral or alkaline pH values and in the presence of oxygen, light, and/or heat, it decomposes into the pharmacologically inactive coloured molecules adrenochrome and adrenolutin (Figure 3). The pH range for the optimal stability of epinephrine in aqueous solution is 3.0-3.8 (Connors *et al.*, 1986). At lower pH values, (-)-epinephrine racemizes into pharmacologically inactive (+)-epinephrine (Figure 3). The rate of acid-catalyzed racemization is extremely slow with ~ 10% racemization into (+)-epinephrine after 10 years of storage at pH 3.5 and 25 °C. In addition, bisulfite, a commonly used antioxidant, reacts with epinephrine to form a pharmacologically inactive epinephrine sulfonate (Figure 3). However, the bisulfite is widely used for the stabilization of epinephrine solutions in autoinjectors because of the slow rate of this reaction compared with the rapid rate of epinephrine oxidation (Connors *et al.*, 1986; Stepensky *et al.*, 2004).

As a powder, epinephrine should be stored in a light-resistant container, and in cool, dry place (USP/NF, 1990).

2.2.2. Pharmacology

Epinephrine is recommended as the drug of choice for the treatment of anaphylaxis due to its ability to relieve the symptoms of anaphylaxis and decrease the release of inflammatory mediators from mast cells and basophils. Epinephrine acts directly on both α- and β-adrenergic receptors. It reverses the hypotension, which is critical for patient survival, through binding to α_1-adrenergic receptors in the smooth muscles of the blood vessels, which induces vasoconstriction, increases peripheral vascular resistance, and reduces mucosal edema. Binding to β_1-receptors in the heart, induces inotropy and chronotropy that results in an increase in the cardiac output and therefore maintains the systolic blood pressure. Also through binding to β_2-receptors in the airways, epinephrine reverses respiratory symptoms due to bronchial obstruction, which is critical for patient survival. In addition, epinephrine at high concentrations decreases the release of inflammatory mediators from mast cells and basophils via binding to β_2-receptors (Hoffman & Lefkowitz, 1996; Simons, 2004).

Epinephrine is extensively metabolized into inactive metabolites after oral administration by the catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver. It is excreted mainly as 3-methoxy-4-hydroxyphenylethylene glycol and 3-methoxy-4-hydroxymandelic acid (Figure 4) (Lefkowitz *et al.*, 1996).

2.3. Limitations of Currently Available Epinephrine Routes of Administration

The recommended intramuscular epinephrine injection can be administered using an epinephrine ampoule/syringe/needle method or epinephrine autoinjectors such as EpiPen® (Dey LP, Nappa, CA) and Twinject® (Verus Pharmaceuticals, Inc. San Diego, CA).

Epinephrine autoinjectors containing either 0.15 mg or 0.3 mg single dose per injection are prescribed for out-of-hospital emergency treatment of anaphylaxis. Epinephrine 0.3 mg dose is suitable for adults and children weighing ≥ 30 kg and epinephrine 0.15 mg dose is suitable for children weighing 15 kg (Sampson et al., 2006; Simons *et al.*, 2002). It is impossible to give an accurate dose to infants and to many children using currently available auto-injectors, which provide only two different premeasured, fixed epinephrine doses (Simons, 2004). In addition, epinephrine autoinjectors, as a self-injectable epinephrine, are underutilized when anaphylaxis occurs (Bock *et al.*, 2001; Gold & Sainsbury, 2000). The drawbacks of epinephrine auto-injectors include: high cost which limits affordability and availability worldwide (Simons, 2005); perceived large size and bulkiness; limitations on repeat dosing (if required) (Korenblat *et al.*, 1999); fear and anxiety associated with the use of needles (Simons, 2004); and dosing errors due to incorrect technique of administration (Gold & Sainsbury, 2000; Sicherer *et al.*, 2000).

Providing an epinephrine ampule, syringe, and needle, as an alternative to an epinephrine autoinjector for infants and young children weighing <15 kg, is

impractical with regard to rapid and accurate dosing by parents and other caregivers who have no medical training (Simons, 2004; Simons *et al.*, 2001a).

Epinephrine by subcutaneous injection, the previously recommended route of administration (Worobec & Metcalfe, 1996), results in delayed absorption and lower plasma levels compared to epinephrine intramuscular injection in the thigh (Simons, 2004; Simons et al., 2001b; Simons *et al.*, 1998).

Epinephrine metered dose inhalers have been investigated as a non-invasive, user-friendly alternative for epinephrine injection for out-of-hospital emergency treatment of anaphylaxis, especially for children. However, most children were unable to inhale sufficient number of doses for treatment of nonrespiratory symptoms (Simons, 2004; Simons *et al.*, 2000).

2.4. Sublingual Route as Alternative Route for Epinephrine Administration

The sublingual route of administration is readily accessible, non-invasive, convenient, and tablets can be removed from the sublingual space, if necessary, to terminate further drug absorption. The high vascularity of the sublingual mucosa facilitates rapid drug absorption directly into the venous circulation through the sublingual and frenular veins (Figure 5) (Netter & Hansen, 2003). Drugs that are absorbed sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form. In addition, they can result in a faster pharmacological response than orally administered drugs

(Bredenberg *et al.*, 2003; Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Motwani *et al.*, 1991; Price *et al.*, 1997).

In humans, the sublingual mucosa has a surface lining consisting of a non-keratinized simple, one layer, epithelium supported by a connective tissue lamina properia, which is nourished by blood vessels (Marieb, 2001). This is similar to the sublingual mucosa of rabbits (Bensley, 1931; Crabbm, 1931), the animal model to be used in this research.

Lipophilic drugs with a low molecular weight such as epinephrine are likely absorbed across the sublingual mucosa into the venous circulation by transcellular diffusion (Birudaraj *et al.*, 2005), a mechanism driven by the concentration gradient (Figure 6) (Sherwood, 2004). Epinephrine would diffuse across the single epithelial cell layer of the mucosa into the interstitial fluid on the basolateral side of the epithelial cells and then into the venous circulation down the concentration gradient according to Fick's law.

The sublingual route is an ideal route for out-of-hospital self-administration of drugs such as nitroglycerine that are used in the treatment of emergency conditions and for drugs that are extensively metabolized following oral administration. It is a promising alternative route for epinephrine administration.

2.5. References

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2.6. Figures

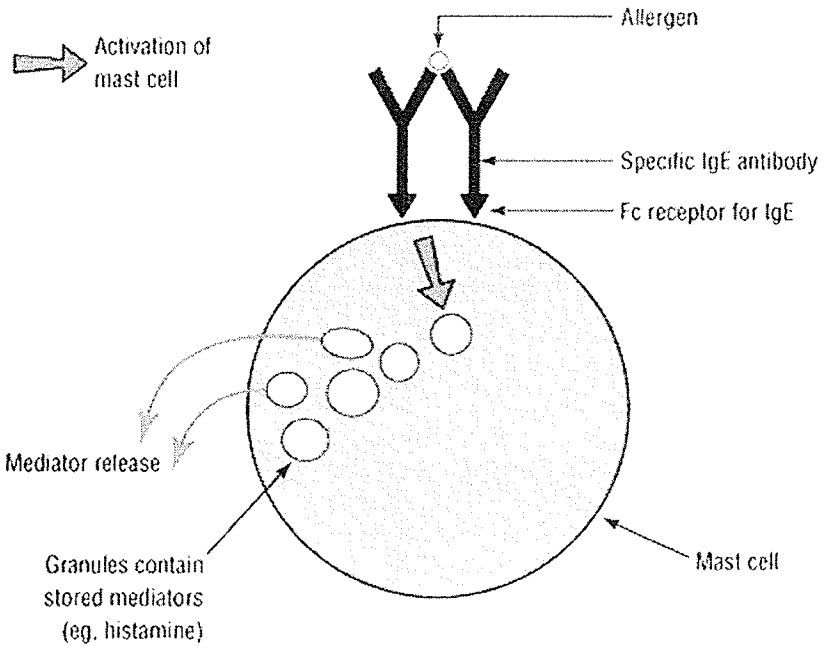

Figure 1: Activation of mast cells by allergen. From: BMJ, 316, Ewan, P. W., ABC of allergies: Anaphylaxis, 1442-1445, © 1998 (Ewan, 1998). Reproduced with permission from BMJ Publishing Group.

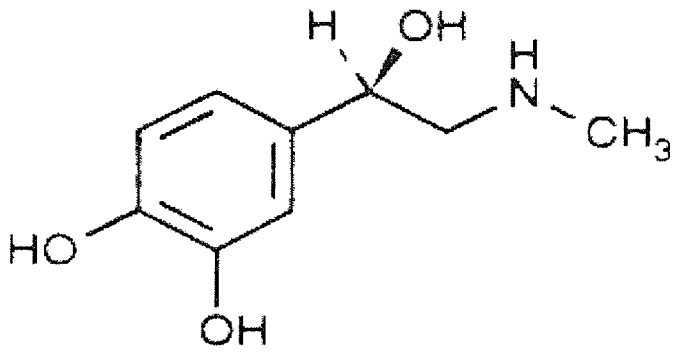

Figure 2: Chemical structure of (-)-epinephrine (USP/NF, 1990)

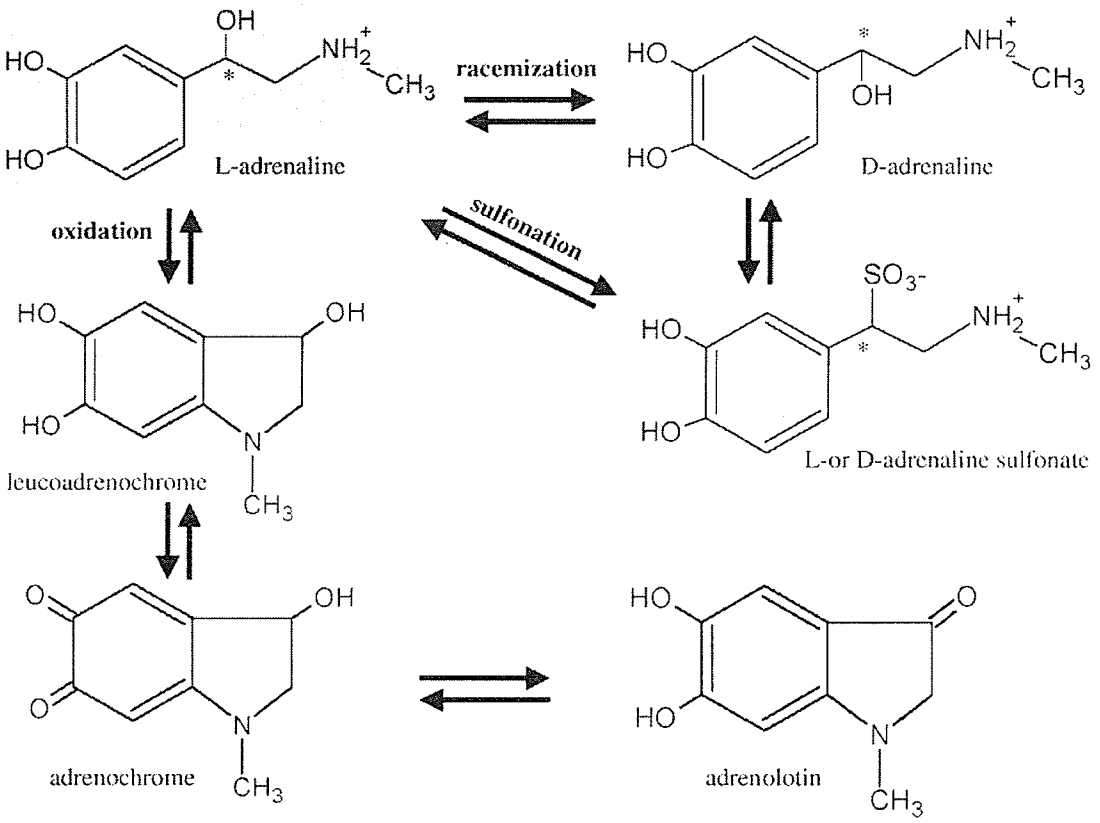

Figure 3: Degradation reactions of (-)-epinephrine. From J Pharm Sci, 93, Stepensky, D., Chorny, M., Dabour, Z., and Schumacher, I., Long-term stability study of L-adrenaline injections: Kinetics of sulfonation and racemization pathways of drug degradation, 969-980, © 2004 (Stepensky et al., 2004). Permission is requested from John Wiley & Sons. Inc.

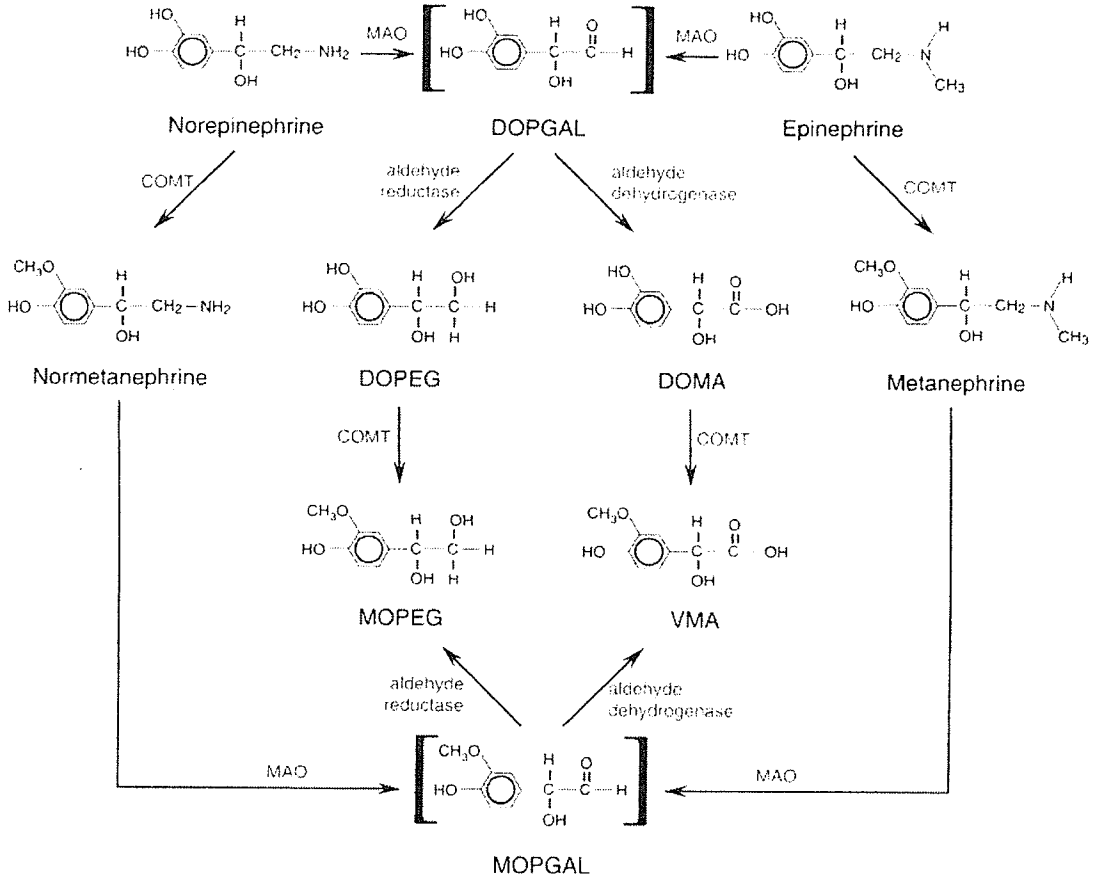

Figure 4: Steps in the metabolic disposition of catecholamines.

Both norepinephrine and epinephrine are first oxidatively deanimated by monoamine oxidase (MAO) to 3,4-dihydroxyphenylglycoaldehyde (DOPGAL) and then either reduced to 3,4-dihydroxyphenylethylene glycol (DOPEG) or oxidized to 3,4-dihydroxymandelic acid (DOMA). Alternatively, they can be initially methylated by catechol-O-methyltransferase (COMT) to normetanephrine and metanephrine, respectively. Most of the products of either type of reaction are then metabolized by the other enzyme to form the major excretory products, 3-methoxy-4-hydroxyphenylethylene glycol (MOPEG or MHPG) and 3-methoxy-4-hydroxymandelic acid (VMA). Free MOPEG is largely converted to VMA. The glycol and, to some extent, the O-methylated amines and the catecholamines may be conjugated to the corresponding sulfates or glucuronides

From: Goodman & Gilman's The Pharmacological Basis of Therapeutics, 9th edition, by Hardman, J.G., Limbird, L.E., Molinoff, P.B., Ruddon, R.W., and Gilman, A.G., © 1996 (Lefkowitz *et al.*, 1996). Permission is requested from McGraw-Hill Companies, Inc.

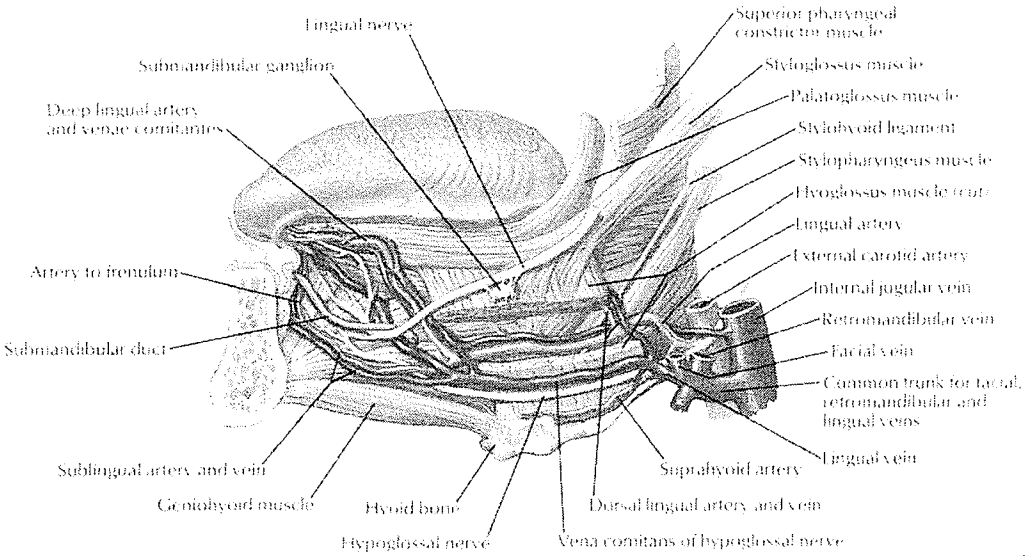

Figure 5: Blood supply to the sublingual cavity. From: Atlas of Human Anatomy, 3rd edition, by Netter, F. and Hansen, J., © 2003 (Netter & Hansen, 2003). Permission is requested from Icon Learning Systems.

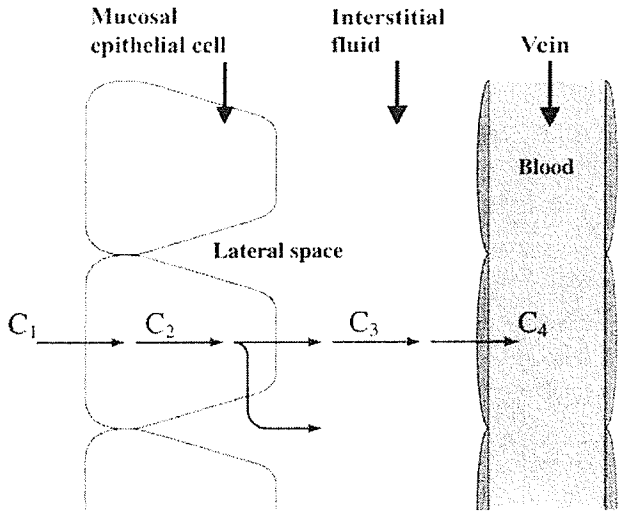

Figure 6: Transcellular absorption of epinephrine from the sublingual cavity into the sublingual veins. C1 epinephrine concentration, which depends on the epinephrine dose and the volume of saliva available; C2 epinephrine concentration within the mucosal epithelial cells; C3 epinephrine concentration in the interstitial fluid; C4 plasma epinephrine concentration. From Bundle: Human Physiology: From Cells to Systems (with CD-ROM and Info-Trac), 5th + Photo Atlas for Biology 5th edition by Sherwood (Sherwood, 2004). © 2004. Adapted and reprinted with permission of Brooks/Cole, a division of Thomson Learning: www.thomsonrights.com.

CHAPTER III: HPLC Analysis of Epinephrine

3.1. Introduction

High Performance Liquid Chromatography (HPLC) was used for the quantification of epinephrine for stability studies of solutions and sublingual tablets, and for the *in vivo* pharmacokinetic studies in the animal model. Ultra violet (UV) detection was used for the detection of high concentrations of epinephrine (micrograms) in aqueous solutions from the stability studies. Electrochemical (EC) detection was used for the detection of low concentrations of epinephrine (picograms) in plasma samples from the pharmacokinetic studies in animals.

3.2. Materials and Methods

An aqueous solution containing 0.1 *M* perchloric acid (Fisher, Fair Lawn, NJ) and 0.1 m*M* sodium metabisulfite (Sigma, St. Louis, MO) to maintain the stability of epinephrine, was used for the preparation of all epinephrine stock solutions and subsequent dilutions, and for desorbing epinephrine from alumina during epinephrine extraction from plasma samples (Hjemdahl, 1987).

3.2.1. HPLC Analysis of Epinephrine from Aqueous Solutions

This method was used for the quantification of epinephrine content in the sublingual tablets for the USP Content Uniformity test, in buffer solutions, in

human saliva, and in sublingual tablets for the epinephrine stability studies. It was also used for the measurement of epinephrine in the autoinjectors, EpiPen® (Dey LP, Nappa, CA), used during the animal studies.

3.2.1.1. HPLC System

The HPLC instrument was a Waters™ (Waters Corp., Milford, MA) component system comprised of a 510 Solvent Delivery System, a 712 WISP autoinjector, and 480 UV Detector. This system was connected to a computer using the Varian Star Integration Software for data analysis. All chromatography was performed on a reversed-phase Nova-Pak® C_{18} column, 3.9x150 mm, 60 nominal pore size, 4 μm spherical particles (Waters Corp., Milford, MA). The wavelength was set at 280 nm (USP/NF, 1990) and the injection volume was 20 μL.

3.2.1.2. Mobile Phase

The mobile phase was composed of 85:15 by volume of buffer:methanol (Fisher, Fair Lawn, NJ), according to USP guidelines (USP/NF, 1990). The buffer was 0.05 *M* sodium phosphate monobasic (Fisher, Fair Lawn, NJ), 519 mg 1-octanesulfonic acid sodium salt monohydrate (Sigma, St. Louis, MO), and 45 mg ethylenediaminetetraacetic acid (EDTA) disodium salt dihydrate (Sigma, St. Louis, MO), adjusted to pH 3.8 using phosphoric acid (Fisher, Fair Lawn, NJ) and filtered using 22 μm nylon membrane filters (Whatman®,

Whatman International Ltd, Maidstone, UK). The flow rate was set at 1.0 mL/min. Under these conditions, epinephrine eluted at 1.9 minutes.

3.2.1.3. Calibration Curve

A stock solution of epinephrine 6 mg/mL was prepared using (-)-epinephrine (+) bitartrate (Sigma, St. Louis, MO), which was diluted to prepare a series of standards containing epinephrine concentrations ranging from 6.125 to 600 μg/mL. A representative HPLC chromatogram of epinephrine is shown in the Appendix, Chromatogram 1.

3.2.2. HPLC Analysis of Epinephrine from Plasma

This method was used for the quantification of epinephrine in plasma samples collected from rabbits following the sublingual administration of the manufactured tablets and the intramuscular epinephrine injection. Epinephrine was extracted from plasma samples before injection into HPLC system using solid phase extraction method (Hjemdahl, 1987). Dihydroxybenzylamine (DHBA) (Sigma, St. Louis, MO) was used as an internal standard.

3.2.2.1. Solid Phase Extraction

Epinephrine extraction was performed according to the procedure recommended by Waters™ and described by Hjemdahl (1987) with some modifications. Alumina was activated by heating 100 g alumina basic, activity grade I (Fisher, Fair Lawn, NJ) in 500 mL 2 *M* hydrochloric acid (Fisher, Fair

Lawn, NJ) to 100 °C for 45 min under continuous and vigorous stirring. The supernatant was removed, and the precipitated alumina was washed, with continuous stirring, sequentially with 250 mL 2 *M* hydrochloric acid at 70 °C for 10 min twice, 500 mL 2 *M* hydrochloric acid at 50 °C for 10 min, and distilled water repeatedly until the wash water reached pH 3.4. The acid-washed alumina was dried and heated to 120 °C for 1 hr and then to 200 °C for 2 hr, and stored at room temperature with desiccant.

A 0.5 mL volume of plasma was added to ~ 25 mg of activated alumina, along with 50 μL of 0.1 m*M* sodium metabisulfite (Sigma, St. Louis, MO), 400 μL of tris buffer, prepared using tris base 121.1 g/L (Sigma, St. Louis, MO) and EDTA 20 g/L, adjusted to pH 8.65 using hydrochloric acid, and precalculated concentrations of DHBA relating to the concentrations used in the calibration curve. The mixture was vortexed for 15 min, to extract epinephrine and DHBA from the plasma samples, and then washed two times with distilled water to remove any plasma components and buffer. The alumina was centrifuged and all remaining water was completely removed by aspiration to prevent subsequent dilution. A 100 μL volume of 0.1 *M* perchloric acid and 0.1 m*M* sodium metabisulfite (1:1) solution was added, vortexed for 5 min to elute epinephrine from alumina. After centrifugation, the supernatant solution was transferred into vials for injection into the HPLC system.

3.2.2.2. HPLC System

The HPLC instrument was a Waters™ (Waters Corp., Milford, MA) component system comprised of a 2690 Alliance separations module and a 2465 electrochemical detector. The potential of the glassy carbon working electrode was set at + 600 mV versus Ag/AgCl reference electrode and the detector sensitivity was set at 10 nA. All chromatography was performed on a reversed-phase Nova-Pak® C_{18} column, 3.9x150 mm, 60 nominal pore size, 4 μm spherical particles (Waters Corp., Milford, MA). The injection volume was 20 μL.

3.2.2.3. Mobile Phase

The mobile phase was composed of 95:15 by volume of buffer:methanol, according to recommendations from Waters™, which conforms to that reported by Ganhao *et al.* (1991), He *et al.* (1997), and Hjemdahl (1987). The buffer was 50 m*M* sodium acetate (Fisher, Fair Lawn, NJ), 20 m*M* citric acid (Fisher, Fair Lawn, NJ), 3.75 m*M* 1-heptanesulfonic acid sodium salt (Sigma, St. Louis, MO), 0.134 m*M* EDTA disodium salt dihydrate (Sigma, St. Louis, MO), and 1 m*M* dibutylamine (Fisher, Fair Lawn, NJ), filtered using 22 μm nylon membrane filters (Whatman®, Whatman International Ltd, Maidstone, UK). The flow rate was set at 1.0 mL/min. Under these conditions, epinephrine and DHBA eluted at 1.9 minutes and 2.5 minutes respectively.

3.2.2.4. Calibration Curves

Two stock solutions of epinephrine, 25 and 250 ng/mL, were prepared using (-)-epinephrine (+) bitartrate (Sigma, St. Louis, MO) and then used to prepare two sets of epinephrine standards ranging from 0.1 to 1.0 ng/mL and from 1.0 to 10.0 ng/mL. A 40 μL volume of DHBA 5 ng/mL (0.2 ng) and a 50 μL volume of DHBA 50 ng/mL (2.5 ng) were used with the low and high range calibration curves, respectively. Representative HPLC chromatograms of epinephrine and DHBA from the low and high range calibration curves are shown in the Appendix, Chromatograms 2 and 3, respectively.

3.3. Results and Discussion

3.3.1. HPLC Analysis of Epinephrine from Aqueous Solutions

Calibration curves were linear with correlation of coefficients (R^2) of > 0.99. The mean peak areas of eight calibration curves collected over 3 months are reported in Table 1 and plotted in Figure 1. The coefficient of variation (CV%) of the system reproducibility at concentrations of 6.125 and 600 μg/mL (n=5 each) were 1.07% and 0.40%, respectively. The intra- and inter-assay CV% were 0.40-0.70% (n=2) and 6.9-3.5% (n=6), respectively.

3.3.2. HPLC Analysis of Epinephrine from Plasma

The extraction recovery from plasma was 84%. The CV% of the system reproducibility in solution at 1.0 ng/mL (n=5) was 0.25%. The detection limit was 5 pg with a CV% of 28.8% (n=2).

The calibration curves were linear with a R^2 of > 0.99. The mean epinephrine concentrations of two low range and eight high range calibration curves, collected over 4 and 9 months respectively, are reported in Tables 2 and 3 and plotted in Figures 2 and 3, respectively. The inter-assay CV% for the low range curve was 0.8-1.5% (n=2). The intra- and inter-assay CV% for the high range calibration curve were 3.5-0.6% (n=2) and 5.0-1.2% (n=6), respectively.

Two calibration curves at two ranges were prepared, instead of one calibration curve with a larger range that covers both ranges, to minimize the peak area differences between epinephrine and DHBA in order to reduce the coefficient of variation in the calculated plasma epinephrine concentrations at the low concentration range of the curve. However, the lower range calibration curve was rarely used because in almost all *in vivo* studies, plasma samples had epinephrine concentrations greater than 1.0 ng/mL.

3.4. References

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3.5. Tables

Table 1: HPLC calibration curve of epinephrine in aqueous solutions

Concentration of Epinephrine (μg/mL)	Peak Area (mg/mL/min)*
6.25	98 ± 6.1
12.5	196 ± 12.5
25	396 ± 25.0
50	763 ± 45.7
100	1508 ± 65.6
200	3020 ± 101.5
300	4575 ± 169.3
600	8974 ± 296.2

*mean ± standard deviation (n = 8)

Table 2: HPLC calibration curve of epinephrine in plasma (low range)

Concentration of Epinephrine (ng/mL)	Calculated Concentration of Epinephrine (ng/mL)*
0.1	0.10 ± 0.001
0.2	0.20 ± 0.006
0.4	0.42 ± 0.026
0.6	0.62 ± 0.017
0.8	0.75 ± 0.014
1.0	1.01 ± 0.015

*mean ± standard deviation (n = 8)

Table 3: HPLC calibration curve of epinephrine in plasma (high range)

Concentration of Epinephrine (ng/mL)	Calculated Concentration of Epinephrine (ng/mL)*
1.0	1.04 ± 0.047
2.0	1.95 ± 0.081
4.0	3.99 ± 0.026
6.0	6.02 ± 0.200
8.0	8.03 ± 0.082
10.0	9.98 ± 0.100

*mean ± standard deviation (n = 8)

3.6. Figures

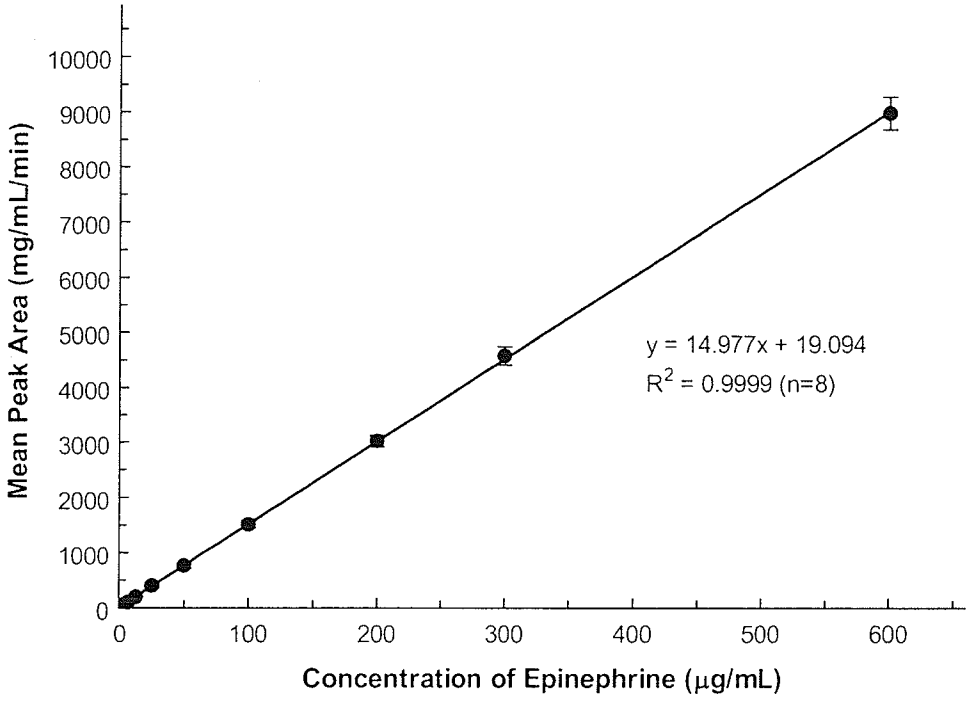

Figure 1: HPLC calibration curve of epinephrine in aqueous solution

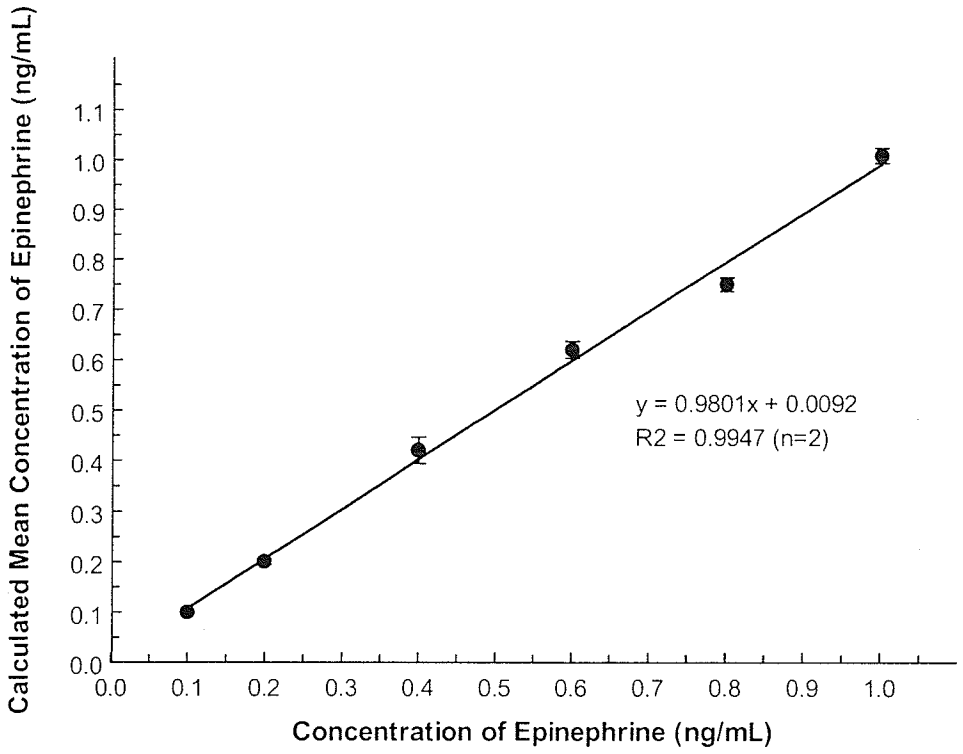

Figure 2: HPLC calibration curve of epinephrine in plasma (low range)

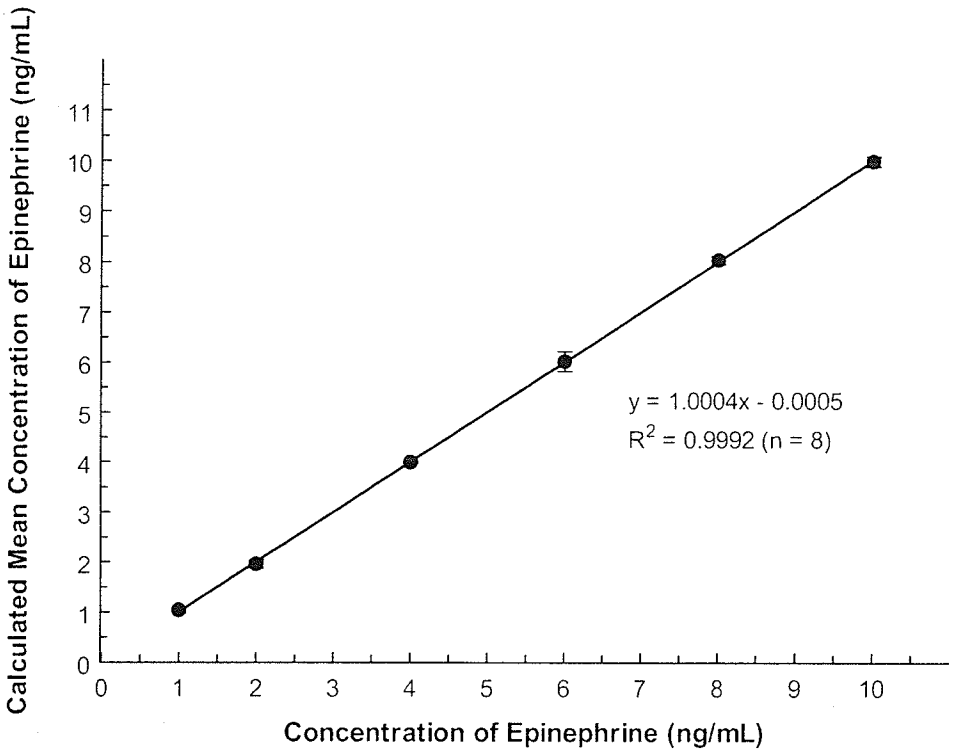

Figure 3: HPLC calibration curve of epinephrine in plasma (high range)

CHAPTER IV: Epinephrine Stability in Human Saliva: Evaluation of Potential for Sublingual Administration

4.1. Abstract

The purpose of this study was to assess the stability of 10 mg epinephrine in buffers pH 5.8 and 7.1, in buffer pH 5.8 with effervescent excipients, and in human saliva. The stability of epinephrine under these conditions was hypothesized to reflect the stability of epinephrine in the sublingual cavity if epinephrine was administered by this route for the emergency treatment of anaphylaxis. Epinephrine 0.1 mL (100 mg/mL) was added to replicate 1.9 mL samples (n=5) of buffer pH 5.8, buffer pH 7.1, buffer pH 5.8 with effervescent excipients (citric acid : sodium bicarbonate, 1:3) and saliva maintained at 37 °C. Replicate samples (n=5) of saliva with no added epinephrine were used as controls. Aliquots of 50 μL were withdrawn at 5, 10, 15, and 20 minutes, diluted and frozen immediately at –20 °C until analyzed for epinephrine content remaining using a high performance liquid chromatography system. Epinephrine content remaining, calculated as doses, were compared statistically using repeated measures two-way ANOVA and Tukey-Kramer tests at a level of significance $p<0.05$. Adding effervescent excipients to the replicate samples of buffer pH 5.8 resulted in mean (± SEM) pH 4.26 ± 0.05. Epinephrine in the control saliva samples was below detection limit. Mean epinephrine doses at baseline and at all sample times in buffer pH 5.8, buffer pH 7.1, buffer pH 5.8

with effervescent excipients and in saliva were not significantly different. Epinephrine is stable in a simulated sublingual environment and would be stable during the sublingual administration for the potential emergency treatment of anaphylaxis.

4.2. Introduction

Epinephrine is the drug of choice for the emergency treatment of anaphylaxis. Currently it is used in an injectable dosage form for this purpose (Lieberman, 2003; McLean-Tooke *et al.*, 2003; Sampson *et al.*, 2006; Simons, 2004). For out-of-hospital emergency treatment of anaphylaxis, epinephrine auto-injectors such as EpiPen®, EpiPen Jr® (Dey LP, Nappa, CA), Twinjet 0.3 mg®, and Twinjet 0.15® (Verus Pharmaceuticals, Inc. San Diego, CA) are prescribed; however, self-injectable epinephrine is underutilized when anaphylaxis occurs (Bock *et al.*, 2001; Gold & Sainsbury, 2000). The drawbacks of epinephrine auto-injectors include: high cost which limits affordability and availability worldwide (Simons, 2005), bulkiness, limitations if repeat dosing is required (Korenblat *et al.*, 1999), fear and anxiety associated with the use of needles (Simons, 2004), and dosing errors due to incorrect administration technique (Gold & Sainsbury, 2000; Sicherer *et al.*, 2000). Oral administration of epinephrine is not feasible due to extensive metabolism by catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver (Lefkowitz *et al.*, 1996). In aqueous solutions, epinephrine is unstable in the

presence of light, oxygen, heat, and neutral or alkaline pH values (Connors *et al.*, 1986).

The sublingual route of administration is a promising non-invasive alternative route for epinephrine administration. Drugs that can be absorbed sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Bredenberg *et al.*, 2003; Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Motwani *et al.*, 1991; Price *et al.*, 1997). The high vascularity of the sublingual mucosa and the low molecular weight of lipophilic epinephrine facilitate its rapid absorption directly into the venous circulation through the sublingual and frenular veins.

Tablets that disintegrate rapidly, orally disintegrating tablets (ODT), would be good candidates for the sublingual administration of epinephrine (Rawas-Qalaji *et al.*, 2006). Epinephrine would be released immediately into the sublingual cavity for absorption through the sublingual mucosa into the systemic circulation by transcellular diffusion (Birudaraj *et al.*, 2005). Effervescent excipients are sometimes used to expedite the tablet disintegration. These excipients, non-medicinal ingredients, can alter the pH of the saliva and may affect the stability of epinephrine.

The pH range for the optimal stability of epinephrine in aqueous solution is 3.0-3.8 (Connors et al., 1986) . Under normal conditions, the saliva pH ranges between 5.8 to 7.1 (Diem *et al.*, 1971).

The required time for the drug to be retained in the sublingual cavity, under the tongue, during the sublingual administration should be less than 5 minutes while drug is continually absorbed into the systemic circulation. Although the contact time between epinephrine and saliva in the sublingual cavity would be minimal, there is no data in the literature evaluating the stability of epinephrine in saliva. If epinephrine is to be administered sublingually for the emergency treatment of anaphylaxis, it is important to determine the stability of epinephrine in saliva and buffers of a pH range simulating various factors that might change the pH of saliva.

The aims of this study were to evaluate the stability of epinephrine in buffer solutions over the normal pH range of human saliva, in buffer solutions with effervescent excipients, and in human saliva.

4.3. Materials and Methods

4.3.1. Materials

(-)-Epinephrine (+) bitartrate was purchased from Sigma-Aldrich (St. Louis, MO, USA). Monobasic potassium phosphate, sodium hydroxide, citric acid anhydrous, sodium bicarbonate, perchloric acid, and sodium metabisulfite were purchased from Fisher Scientific (Nepean, ON, Canada).

4.3.2. Methods

Five replicates of 1.9 mL phosphate buffer samples were prepared at pH 5.8 (A) and pH 7.1 (B), using potassium phosphate monobasic and sodium

hydroxide according to the USP (USP/NF, 1990b). Effervescent excipients, citric acid anhydrous and sodium bicarbonates (ratio 3:1), were added to another five replicates of 1.9 mL phosphate buffer samples at pH 5.8 (C). Five replicates of 1.9 mL human saliva samples were collected. The volunteer was asked not to eat or drink any acidic, alkaline, or spicy food or beverages and to rinse the mouth before saliva collection. The saliva was collected into a beaker maintained on ice during the collection time period. All the buffer and saliva samples were maintained at 37 °C in a water bath for 5 minutes before the addition of epinephrine. All test samples were spiked with epinephrine 0.1 mL (100 mg/mL), vortexed, then returned immediately to the 37 °C water bath. The time when epinephrine was added was recorded as baseline time. Five replicates of blank saliva samples, not spiked with epinephrine, were used as control during the study. Aliquots of 50 μL were withdrawn from all samples at 5, 10, 15, and 20 minutes. The 50 μL samples were diluted to 2.0 mL, using a solvent of 0.1 M perchloric acid and 0.1 mM sodium metabisulfite (Hjemdahl, 1987), and frozen immediately at –20 °C after collection until analysis for epinephrine content using a reverse phase high performance liquid chromatography system (Waters Corp., Milford, MA) with ultra violet detection (USP/NF, 1990a).

4.3.3. Data Analysis

The epinephrine dose remaining in each of the different pH buffers and saliva replicates at the different times were compared using repeated measures two-way ANOVA and Tukey-Kramer tests using NCSS Statistical

Analysis Software (NCSS, Kaysville, UT). Differences were considered to be significant at $p < 0.05$.

4.4. Results

Mean (± SEM) pH measured in the five replicates of buffer pH 5.8 samples after the addition of effervescent excipients was 4.26 ± 0.05. Endogenous epinephrine concentrations in the control saliva samples were below the limit of detection. The mean (± SEM) epinephrine dose remaining in the five replicates of each buffer samples and saliva samples are shown in Table I. The mean (± SEM) epinephrine dose remaining in buffer A, B, and C, and in saliva did not differ significantly from each other at any time and from the stock epinephrine dose at zero time.

4.5. Discussion

The pH of the solvent medium is one of the several factors that can influence epinephrine stability. The pH range for optimal epinephrine stability in aqueous solution is between 3.0 and 3.8. At lower pH values the rate of epinephrine racemization increases and at higher pH values the rate of epinephrine oxidation increases (Connors et al., 1986). The range of pH in human saliva, 5.8 to 7.1, is higher than the optimal pH range for epinephrine stability, therefore oxidation might be the main pathway for epinephrine decomposition to the inactive pharmacological forms, adrenochrome and adrenolutin (Stepensky *et al.*, 2004). Saliva does not contain catechol-O-

methyltransferase or monoamine oxidase that convert epinephrine into its inactive metabolites, 3-methoxy-4-hydroxyphenylethylene glycol and 3-methoxy-4-hydroxymandelic acid (Lefkowitz et al., 1996).

In this study it was confirmed that epinephrine is stable in buffers at the same pH range as human saliva and is also stable in human saliva for at least twenty minutes, thereby exceeding the expected duration of time, < 5 min, required for sublingual absorption.

The addition of anhydrous citric acid and sodium bicarbonate can change the pH of saliva. A ratio of 3:1 for citric acid anhydrous : sodium bicarbonate was selected as a representative ratio of citric acid and sodium bicarbonate to form an effervescent product.

Epinephrine was not detected in the control saliva samples because endogenous epinephrine is not secreted in the salivary secretions (Diem et al., 1971).

4.6. Conclusions

Epinephrine is stable in aqueous solutions at non-optimal pH and in human saliva for at least 20 minutes. The sublingual route is feasible for the sublingual administration of epinephrine.

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4.8. Tables

Table 1. Epinephrine dose remaining in different buffers and human saliva*†

Time (min)	Remaining epinephrine dose (mg)			
	A	B	C	Saliva
5	9.37 ± 0.23	10.04 ± 0.25	10.43 ± 0.39	10.52 ± 0.38
10	9.69 ± 0.32	9.86 ± 0.18	10.06 ± 0.20	10.47 ± 0.27
15	9.38 ± 0.31	10.08 ± 0.21	10.07 ± 0.11	10.21 ± 0.13
20	9.41 ± 0.26	9.41 ± 0.50	10.17 ± 0.10	10.12 ± 0.09

*mean ± SEM (n=5); A: phosphate buffer pH 5.8; B: phosphate buffer pH 7.1; C: phosphate buffer pH 5.8 with citric acid and sodium bicarbonate (ratio 3:1)

†$p>0.05$

CHAPTER V: Fast-Disintegration Sublingual Tablets: Effect of tablet Dimensions on Tablet Characteristics*

5.1. Abstract

The purpose of this study was to evaluate the effect of changing dimensions on the hardness (H), disintegration time (DT), and wetting time (WT) of fast-disintegrating epinephrine tablets for sublingual administration as potential emergency treatment of anaphylaxis. Tablet formulations, I and II, containing 0% and 10% epinephrine bitartrate, respectively, and weighing 150 mg were prepared by direct compression. Formulations were compressed at a range of forces using an 8/32" die with concave punches (CP); a 10/32" and an 11/32" die with CP and flat punches (FP). Tablet weight variation, content uniformity, flowability, thickness, H, DT, and WT were measured. The 8/32", 10/32", and 11/32" dies resulted in tablet thickness ranges of 0.25"-0.19", 0.17"-0.1", and 0.16"-0.08", respectively. The DT and WT using the 8/32" die were ≤10 sec and ≤30 sec, respectively, at H ≤5.4 ± 0.2 kg for formulation I, and H ≤5.4 ± 0.3 kg for formulation II. The DT and WT were ≤10 sec and ≤30 sec, respectively, using 10/32" die/CP, 10/32" die/FP, 11/32" die/CP, and 11/32" die/FP at H ≤6.2 ± 0.6 kg, ≤6.8 ± 0.4 kg, ≤4.9 ± 0.1 kg, and ≤7.2 ± 0.3 kg, respectively, for formulation I. For formulation II, the DT and WT were ≤10 sec and ≤30 sec, respectively, when

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* Accepted by Drug Dev. Ind. Pharm. (2006), Rawas-Qalaji, M., Simons, E., and Simons, K., Fast-Disintegrating Sublingual Tablets: Effect of Tablet Dimensions on Tablet Characteristics, permission to be obtained from Taylor & Francis Group, LLC

H <4 kg. No difference in DT and WT was observed between concave and flat tablets. The 11/32" and 10/32" dies resulted in more ideal tablet dimensions for sublingual administration, but H must be maintained <4 kg to ensure rapid DT and WT.

5.2. Introduction

Fast-disintegrating and fast-dissolving tablets became more popular as novel delivery systems for drug administration. They are more convenient for children, elderly patients, patients with swallowing difficulties, and in the absence of potable liquids (Allen, 2003; Fu *et al.*, 2004). In addition, sublingual administration of drugs formulated using these tablets can result in a faster pharmacological response than using oral tablets (Bredenberg *et al.*, 2003; Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Price *et al.*, 1997) and bypass the gastrointestinal and the hepatic first pass metabolic processes (Lefkowitz *et al.*, 1996). These tablets could be good candidates for the treatment of emergency conditions via the sublingual route of administration and for drugs that are extensively metabolized following oral administration.

Epinephrine is the drug of choice for the emergency treatment of anaphylaxis. It is available in injectable dosage forms (Lieberman, 2003; McLean-Tooke *et al.*, 2003; Sampson *et al.*, 2006; F. E. R. Simons, 2004). It is extensively metabolized after oral administration by catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver (Lefkowitz *et al.*, 1996). In aqueous

solutions, epinephrine is unstable in the presence of light, oxygen, heat, and neutral or alkaline pH values (Connors *et al.*, 1986). Feasibility studies in humans (K. J. Simons *et al.*, 2004) and animals (Rawas-Qalaji *et al.*, 2006a) have shown that epinephrine can be absorbed sublingually. Epinephrine is available as very water-soluble hydrochloride and bitartrate salts.

Extremely fast tablet disintegration would be required to expedite the availability of epinephrine for rapid absorption by the sublingual mucosa blood vessels. Various techniques can be used to formulate fast-disintegrating or dissolving tablets (Allen, 2003; Fu *et al.*, 2004). In this study, direct compression was used to manufacture fast-disintegrating sublingual epinephrine tablets containing a super-disintegrant, to circumvent the use of heat or moisture during the manufacturing processes. The appropriate tablet dimension and shape that demonstrates an ideal fast-disintegrating tablet's characteristics is required for manufacturing epinephrine tablets for sublingual administration as potential emergency treatment of anaphylaxis.

Tablets intended for sublingual administration may require dimensions different from those tablets for oral administration. Sublingual tablets should have either very small dimensions such as nitroglycerine tablets or be thin and flat to fit comfortably into the sublingual cavity. In contrast to tablets for oral administration, changes in sublingual tablet dimensions could affect the disintegration and wetting times as the excipients (non-medicinal ingredients) are replaced with increasing percentages of medication.

The aim of this study was to evaluate the effect of changing tablet dimensions, by modifying diameter, thickness, and shape, on tablet hardness, disintegration time, and wetting time while retaining constant tablet weight, but adjusting medication : excipients ratios.

5.3. Materials and Methods

5.3.1. Materials

(-)-Epinephrine (+) bitartrate was purchased from Sigma-Aldrich (St. Louis, MO). It was used because it was readily obtainable as the pure L-isomer, the pharmacologically active form. Ceolus ® PH-301 (microcrystalline cellulose) with a mean particle size of 50 μm was supplied by Asahi Kasei Chemicals Corp (Tokyo, Japan) and low-substituted hydroxypropyl cellulose (LH11) with a mean particle size of 50 μm was supplied by Shin-Etsu Chemical Co (Tokyo, Japan). Magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ).

5.3.2. Preparation of Tablets

Two tablet formulations, I and II containing 0% and 10% (15 mg) of epinephrine bitartrate respectively, were prepared by direct compression (Table 1). The total weight of the compressed tablets was maintained at 150 mg. Tablet formulations were prepared by mixing the precalculated weight of epinephrine bitartrate with the total quantity of microcrystalline cellulose and two-thirds of the quantity of low-substituted hydroxypropyl cellulose by using a three dimensional manual mixer (Inversina ®, Bioengineering AG, Switzerland). The microcrystalline

cellulose : low-substituted hydroxypropyl cellulose ratio in each of the final tablet formulations was always maintained at 9:1 (Bi *et al.*, 1999; Bi *et al.*, 1996; Ishikawa *et al.*, 2001; Watanabe *et al.*, 1995). All of the magnesium stearate and the remaining one-third of the quantity of low-substituted hydroxypropyl cellulose were added to the powder and mixed for 30 seconds, as a running powder, to achieve external positioning of the low-substituted hydroxypropyl cellulose and the magnesium stearate. To achieve rapid and complete tablet disintegration, it is very important that the low-substituted hydroxypropyl cellulose is positioned both internally and externally (Sheth *et al.*, 1980).

Each tablet formulation was assessed for flowability by measuring the angle of repose and then compressed at a pre-selected range of compression forces (CF). An 8/32" die with concave upper and lower punches (CP), and 10/32" and 11/32" dies with CP and flat, scored face, bevel edge upper punch and a bevel edge lower punch (FP) were used during compressing the tablet formulations. The various tablet shapes and dimensions were compressed using a Manesty ® – F3 single-punch tablet press machine (Liverpool, UK).

5.3.3. Evaluation of Tablet Characteristics

Each batch of 200 tablets was collected into a stainless steel beaker. Tablet weight variation and drug content uniformity was measured using USP methods and criteria (USP/NF, 2003). Six tablets were selected randomly from each formulation batch and tested for tablet hardness, disintegration time, and

wetting time. The mean ± standard deviation (SD) and percentage of coefficient of variation (CV%) were calculated.

5.3.3.1. Thickness (T)

The T of both concave and flat tablets was measured at the center of the tablet using a dial calliper (Hempe Manufacturing Co., Inc., New Berlin, WI).

5.3.3.2. Hardness (H)

The H or the crushing tolerance of tablets was measured using an Erweka ® hardness tester (Heusenstamm, Germany).

5.3.3.3. Disintegration Time (DT)

A relatively simple method with rigorous conditions was developed (Rawas-Qalaji *et al.*, 2006b) to evaluate the DT of rapidly disintegrating tablets. Each individual tablet was dropped into a 10 ml glass test tube (1.5 cm diameter) containing 2 ml distilled water, and the time required for complete tablet disintegration was observed visually and recorded using a stopwatch. The visual inspection was enhanced by gently rotating the test tube at a 45° angle, without agitation, to distribute any tablet particles that might mask any remaining undisintegrated portion of the tablets.

5.3.3.4. Wetting Time (WT)

Tablet WT was measured by a procedure modified from that reported by Bi *et al* (1996). The tablet was placed at the center of 2 layers of absorbent paper fitted into a rectangular plastic dish (11 cm X 7.5 cm). After the paper was thoroughly wetted with distilled water, excess water was completely drained out of the dish. The time required for the water to diffuse from the wetted absorbent paper throughout the entire tablet was observed visually and recorded using a stopwatch.

5.3.4. Data Analysis and Curve Fitting

All results were reported as mean ± SD (n=6) and analyzed by plotting H versus CF; DT and WT versus H. The relationships were fitted to appropriate equations using Axum 5.0C (MathSoft, Inc.) and NCSS (NCSS, Kaysville, Utah) softwares. The constants of each equation and the correlation of fit (R^2) were calculated using NCSS and Excel 2000 (Microsoft Corporation) softwares.

5.4. Results and Discussion

The powders from both formulation I and II resulted in very good mixing, flowability, and compressibility characteristics. The angles of repose for formulation I and II were 30° and 40°, respectively (Wadke & Jacobson, 1980). Tablets manufactured from each formulation were within USP specifications for weight variation and drug content uniformity (USP/NF, 2003).

5.4.1. Hardness

The H values of the compressed tablets resulting from a series of linearly increasing CF using different die's sizes and punches' shapes for formulation I and II are illustrated in Figure 1. It was shown previously that a linear increase in the CF resulted in an exponential increase in the tablet H (Rawas-Qalaji *et al.*, 2006b). At lower CF, elastic deformation would be the main form of microcrystalline cellulose particles rearrangement. Once the CF exceeded the elastic deformation forces, plastic deformation would be the more dominant form of microcrystalline cellulose particles rearrangement (Marshall, 1986), which would result in a low tablet porosity and harder tablet compact that would affect or even limit tablet disintegration and wetting (Bi *et al.*, 1999; Bi *et al.*, 1996; Sugimoto *et al.*, 2001; Watanabe *et al.*, 1995). Similar results were obtained in this study, using the three different die sizes, with both concave and flat-scored punches. The correlation between CF and H can be described by Equation 1, where X is CF and Y is H. The equation constants (a and b) for the different tablet dimensions and shapes of both formulations are shown in Table 2.

$$Y = ae^{bX} \qquad (1)$$

As the die size was increased to produce thinner, larger diameter tablets, lower CF were required to achieve a comparable range of H (mean ± SD, 0.9±0.1-12.0±0.4 kg). Using dies with larger diameter would increase the contact points between the powder surface and the punches, and result in a thinner powder layer in the die, requiring lower CF. Also, tablets compressed using CP

required higher CF than FP to achieve a comparable range of H, as more force may be required at the perimeter of the tablets to form the concave shape.

The exponential increase in the tablet H, following the linear increase in the CF was more dramatic with the use of dies of larger diameters, 10/32" and 11/32", resulting in thinner tablets. This dramatic increase in the tablet H, despite the lower CF required when compared to the 8/32" die, was shown by the increment of the slope (b) values (Table 2), and the smaller range of CF required to compress these tablets as shown in Figure 1. The thinner powder layer resulted in fewer particles to be compacted and fewer void spaces available for particles rearrangement per a unit range of the tablet diameter, which resulted in more plastic than elastic deformation.

The resulting tablet thickness for a series of increasing CF values using the 8/32", 10/32", and 11/32" dies ranged from 0.25" to 0.19", 0.17" to 0.1", and 0.16" to 0.08" respectively. Tablets compressed using 8/32" die were considered to be too thick for use as sublingual tablets. The dimensions of tablets compressed using the 10/32" and 11/32" dies were deemed to be more ideal for sublingual administration.

5.4.2. Disintegration and Wetting Time

For fast-disintegrating or fast-dissolving tablets, the standard apparatus and procedure specified in the USP (USP/NF, 1990a, 1990b) cannot measure accurately the differences in the disintegration times of these tablets. Instead, a relatively simple method was used in this study as previously

described, to evaluate the DT of fast-disintegrating tablets intended for the sublingual administration (Rawas-Qalaji *et al.*, 2006b).

Salivary secretions in humans can vary between 0.35-1.0 ml/min under normal conditions. These salivary volumes are very small in comparison with the large volume of solution (900 mL) used in the USP disintegration test (USP/NF, 1990b). It was determined that the wetting test of Bi *et al* (1996) compared favourably with sublingual salivary volumes and sublingual conditions *in vivo*. While not an official USP test, it can predict the tablet wettability in the presence of minimal amounts of liquid and more ideally represents the conditions of epinephrine tablet disintegration in the sublingual cavity.

The DT and WT values versus H, for both formulations with different diameters and shapes, are shown in Figures 2 and 3 respectively. The maximum H for both formulations that resulted in DT ≤ 10 sec and WT ≤ 30 sec are shown in Table 3.

For formulation I, all tablets with different shapes and dimensions resulted in short DT (≤ 10 sec) and WT (≤ 30 sec) at a wide range of H (Figures 2a and 3a respectively). The maximum H ± SD at which the various tablets resulted in fast disintegration and wetting was relatively similar and ranged between 4.9 ± 0.1 kg and 7.2 ± 0.3 kg (Table 3).

For formulation II, the tablets with 8/32" diameter also resulted in DT ≤ 10 sec and WT ≤ 30 sec at a wide range of H (Figures 2b and 3b) and the maximum H at which these tablets resulted in fast disintegration and wetting was similar to formulation I (Table 3). Although there was no dramatic difference in

the DT and WT between formulations I and II, these tablets were considered less suitable for sublingual administration than 10/32" and 11/32" diameter-tablets.

The DT and WT for formulation II tablets with 10/32" and 11/32" diameters increased dramatically at higher H, so to retain the same DT and WT, less CF resulting in lower H should be used (Table 3). The difference in the DT and WT between 10/32" and 11/32" diameter-tablets and 8/32" diameter-tablets at higher H in the presence of an epinephrine bitartrate load in the formulation is possibly due to the effect of a higher number of bonds formed during compaction of these thinner 10/32" and 11/32" diameter-tablets, which would affect the type of deformation. A closer particle arrangement during the compaction occurred, due to fewer particles and fewer void spaces available for compaction per unit range of the tablet diameter as the powder load in the die cavity becomes thinner. The low compressibility of epinephrine bitartrate leads to the formation of more irreversible bonds between particles, so plastic deformation was probably more dominant, resulting in longer DT and WT for these tablets. In addition, the significant decrease in the tablet porosity, due to the incorporation of epinephrine bitartrate (Rawas-Qalaji *et al.*, 2006b) and the fewer void spaces available between particles as described previously, would also adversely affect the DT and WT. These results indicate that loading epinephrine bitartrate into formulation II resulted in a greater negative impact on the DT and WT of 10/32" and 11/32" diameter-tablets than on the 8/32" diameter-tablets at H > 4 kg (Figures 2b and 3b respectively).

There was a general increase in the DT and WT of tablets from formulation II when compared to formulation I, especially when the H was > 4 kg. The delay was more dramatic with larger diameter-tablets and the effect of loading epinephrine bitartrate in formulation II on the tablets characteristics has been reported previously (Rawas-Qalaji *et al.*, 2006b).

For 10/32" and 11/32" dies, changing from concave punches to flat punches had no effect on DT and WT. This could be due to the small difference in the tablet surface area and dimensions between the 10/32" and 11/32" diameter-tablets.

5.5. Conclusions

Tablets containing epinephrine bitartrate with dimensions and shapes suitable for sublingual administration can be formulated without adversely affecting fast disintegration and wetting times, and could have the potential for the emergency treatment of anaphylaxis. The sublingual bioavailability of epinephrine from this tablet formulation is being evaluated in the validated rabbit model.

5.6. References

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5.7. Tables

Table 1. Composition of tablet formulations I and II*

	Tablet Formulations	
Ingredient Weight %	I	II
Epinephrine bitartrate	-	10 %
Microcrystalline cellulose (PH-301)	88.2 %	79.2 %
Low-Substituted hydroxypropyl cellulose (LH11)	9.8 %	8.8 %
Magnesium stearate	2 %	2 %

*Tablet weight was 150 mg.

Table 2. Correlation constants, a and b, for formulations I and II*

	CP						FP			
	8/32"		10/32"		11/32"		10/32"		11/32"	
Constants for	a	b	a	b	a	b	a	b	a	b
I	$2x10^{-5}$	0.37	$2x10^{-6}$	0.57	$1x10^{-8}$	0.82	$1x10^{-7}$	0.77	$3x10^{-7}$	0.72
II	$5x10^{-6}$	0.41	$1x10^{-7}$	0.70	$1x10^{-6}$	0.62	$7x10^{-8}$	0.78	$2x10^{-7}$	0.74

*CP indicates concave punches; FP, flat-scored punches

Table 3. The maximum hardness at which tablets from formulations I and II resulted in disintegration time ≤ 10 sec and wetting time ≤ 30 sec*

	CP						FP			
	8/32"		10/32"		11/32"		10/32"		11/32"	
Formulation	H	CV	H	CV	H	CV	H	CV	H	CV
I	5.4±0.2	4.2	6.2±0.6	10.3	4.9±0.1	2.5	6.8±0.4	2.2	7.2±0.3	4.5
II	5.4±0.3	5.3	3.8±0.3	8.7	3.2±0.2	5.5	3.3±0.2	2.2	3.8±0.3	6.6

*CP indicates concave punches; FP, flat-scored punches; H, mean ± SD tablet hardness (kg); CV, coefficient of variation (%)

5.8. Figures

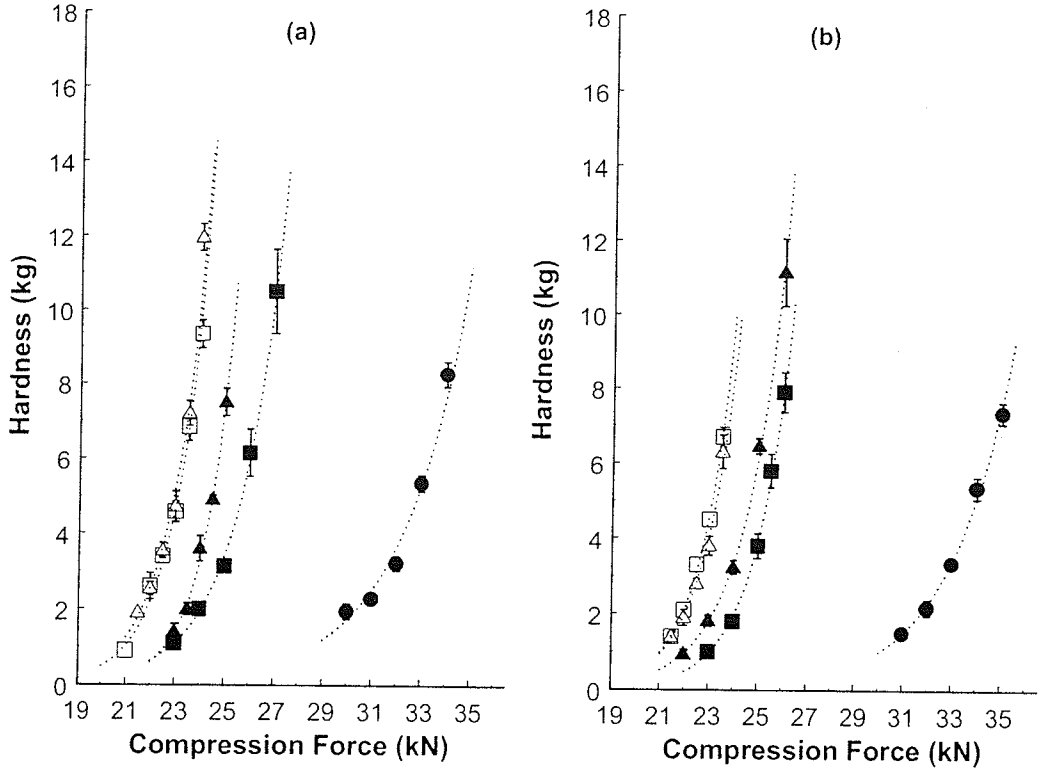

Figure 1: Effect of increasing compression force on tablet hardness for formulation I (panel a) and II (panel b). Symbols: closed: concave punches, open: flat punches; circle: 8/32" die; square: 10/32" die; triangle: 11/32" die. Data are represented as mean ± SD (n = 6). R^2 is ≥ 0.97 in all formulations.

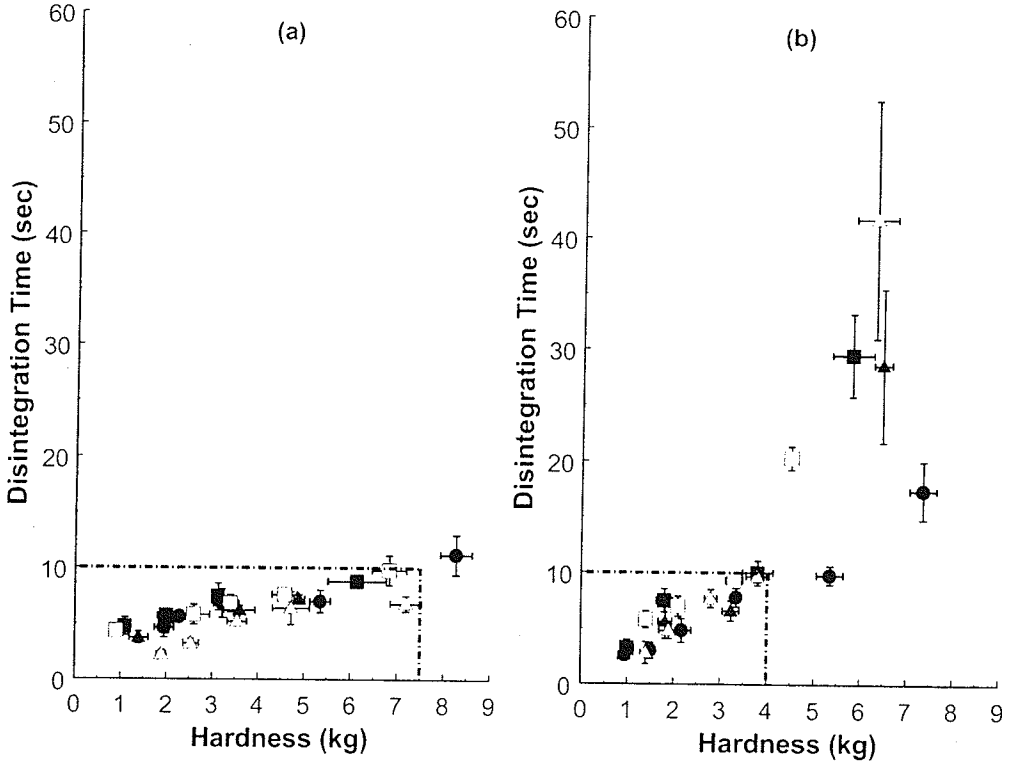

Figure 2: Relationship between tablet hardness and disintegration time for formulation I (panel a) and II (panel b). Symbols: closed: concave punches, open: flat punches; circle: 8/32" die; square: 10/32" die; triangle: 11/32" die. Data are represented as mean ± SD (n = 6).

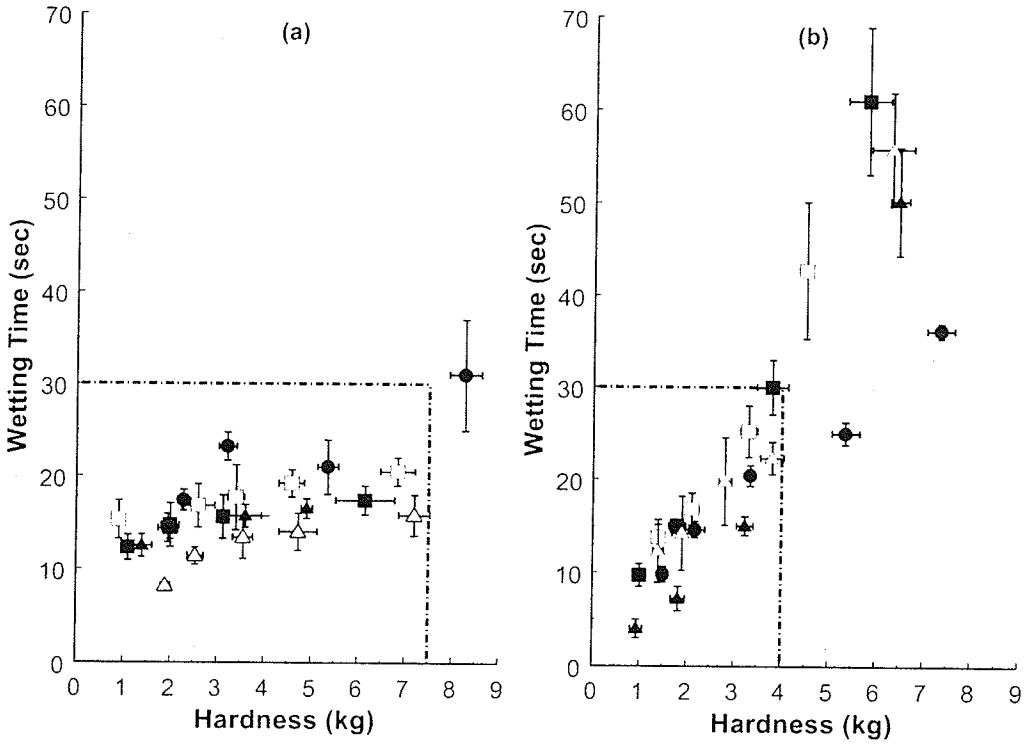

Figure 3: Relationship between tablet hardness and wetting time for formulation I (panel a) and II (panel b). Symbols: closed: concave punches, open: flat punches; circle: 8/32" die; square: 10/32" die; triangle: 11/32" die. Data are represented as mean ± SD (n = 6).

CHAPTER VI: Fast-Disintegrating Sublingual Tablets: Effect of Epinephrine Load on Tablet Characteristics*

6.1. Abstract

The aim of this study was to evaluate the effect of increasing epinephrine load on the characteristics of fast-disintegrating sublingual tablets for the potential emergency treatment of anaphylaxis. Four tablet formulations, A, B, C, and D, containing 0%, 6%, 12%, and 24% of epinephrine bitartrate respectively, and microcrystalline cellulose : low-substituted hydroxypropyl cellulose (9:1), were prepared by direct compression, at a range of compression forces. Tablet weight variation, content uniformity, hardness, disintegration time, wetting time, and friability were measured for each formulation at each compression force. All four tablet formulations at each compression force were within the USP limits for weight variation and content uniformity. A linear increase in compression force resulted in an exponential increase in hardness for all formulations, a linear increase in disintegration and wetting times of A, and an exponential increase in disintegration and wetting times of B, C, and D. At a mean ± SD hardness of ≥ 2.3 ± 0.2 kg, all tablet formulations passed the USP friability test. At a mean ± SD hardness of ≤ 3.1 ± 0.2 kg, all tablet formulations resulted in disintegration and wetting times of < 10 sec and < 30 sec, respectively. Tablets with drug loads

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from 0% to 24% epinephrine can be formulated with hardness, disintegration times, and wetting times suitable for sublingual administration.

6.2. Introduction

Tablets that disintegrate or dissolve rapidly in the patient's mouth are convenient for young children, the elderly and patients with swallowing difficulties, and also in situations where potable liquids are not available. For these formulations, the small volume of saliva is usually sufficient to result in tablet disintegration in the oral cavity. The medication can then be absorbed partially or entirely into the systemic circulation from blood vessels in the sublingual mucosa, or it can be swallowed as a solution to be absorbed from the gastrointestinal tract. The sublingual route usually produces a faster onset of action than orally ingested tablets and the portion absorbed through the sublingual blood vessels bypasses the hepatic first pass metabolic processes (Birudaraj *et al.*, 2005; Ishikawa *et al.*, 2001a; Price *et al.*, 1997).

Epinephrine is the drug of choice for the treatment of anaphylaxis worldwide (Joint Task Force on Practice Parameters; American Academy of Allergy Asthma and Immunology; American College of Allergy Asthma and Immunology; Joint Council of Allergy Asthma and Immunology, 2005; Lieberman, 2003; F. E. R. Simons, 2004). It is available as an injectable dosage form in ampules or in autoinjectors. In aqueous solutions, epinephrine is unstable in the presence of light, oxygen, heat, and neutral or alkaline pH values (Connors *et al.*, 1986). Feasibility studies in humans (K. J. Simons *et al.*, 2004) and animals (Rawas-

Qalaji *et al.*, 2006) have shown that epinephrine can be absorbed sublingually. The optimal sublingual epinephrine dose for the treatment of anaphylaxis is unknown. Epinephrine is available as very water-soluble hydrochloride and bitartrate salts. Epinephrine bitartrate was used in this study because it was readily obtainable as the pure L-isomer, the pharmacologically active form.

Various techniques can be used to formulate rapidly-disintegrating or dissolving tablets (Allen, 2003; Fu *et al.*, 2004). Direct compression, one of these techniques, requires the incorporation of a super-disintegrant into the formulation, or the use of highly water-soluble excipients to achieve fast tablet disintegration. Direct compression does not require the use of water or heat during the formulation procedure and is the ideal method for moisture- and heat-labile medications. However, the direct compression method is very sensitive to changes in the type and proportion of excipients and in the compression forces, when used to achieve tablets of suitable hardness without compromising the rapid disintegration characteristics. Unique packaging methods such as strip-packaging, could be used to compensate for the problem of extreme friability of rapidly disintegrating tablets. Watanabe *et al* (1995) and Bi *et al* (1996) were the first to evaluate the ideal excipient proportions and other related parameters using a super-disintegrant, in order to formulate durable fast-disintegrating tablets for oral administration. The effect of a wide range of microcrystalline cellulose : low-substituted hydroxypropyl cellulose ratios on the tablet characteristics was studied. A ratio of 9:1 and 8:2 resulted in greater tablet hardness in association with shorter disintegration and wetting times. Based on

the results obtained by Watanabe *et al* and Bi *et al*, a microcrystalline cellulose : low-substituted hydroxypropyl cellulose ratio of 9:1 was selected as the optimal ratio to formulate and test the development of epinephrine tablets for sublingual administration. Extremely fast tablet disintegration would be required to enhance the release of epinephrine from tablets for rapid absorption by the sublingual mucosa blood vessels.

It was hypothesized that epinephrine could be formulated into fast-disintegrating tablets for sublingual administration as potential emergency treatment of anaphylaxis. This could be achieved by selecting the appropriate pharmaceutical excipients in the correct proportion, in combination with optimal manufacturing techniques and compression parameters. The aim of this study was to evaluate the effect of increasing epinephrine bitartrate load on the hardness, disintegration time, and wetting time of a fast-disintegrating tablet formulation.

6.3. Materials and Methods

6.3.1. Materials

(-)-Epinephrine (+) bitartrate, (-)-3,4-dihydroxy-α-[(methylamino)methyl]benzyl alcohol (+)-tartrate (1:1) salt, was purchased from Sigma-Aldrich (St. Louis, MO). Ceolus ® PH-301 (microcrystalline cellulose) with a mean particle size of 50 μm was supplied by Asahi Kasei Chemicals Corp (Tokyo, Japan) and low-substituted hydroxypropyl cellulose (LH11) with a mean

particle size of 50 μm was supplied by Shin-Etsu Chemical Co (Tokyo, Japan). Magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ).

6.3.2. Preparation of Tablets

Four tablet formulations, A, B, C, and D containing 0%, 6%, 12%, and 24% of epinephrine bitartrate, equivalent to 0, 5, 10, and 20 mg of epinephrine respectively, were prepared by direct compression (Table 1). The total weight of the compressed tablets was maintained at 150 mg. These tablets were prepared by mixing the precalculated weight of epinephrine bitartrate with the total quantity of microcrystalline cellulose and two-thirds of the quantity of low-substituted hydroxypropyl cellulose by using a three dimensional manual mixer (Inversina ®, Bioengineering AG, Switzerland). The microcrystalline cellulose : low-substituted hydroxypropyl cellulose ratio in each of the final tablet formulations was always maintained at 9:1 (Bi *et al.*, 1999; Bi et al., 1996; Ishikawa *et al.*, 2001b; Watanabe et al., 1995). All of the magnesium stearate and the remaining one-third of the quantity of low-substituted hydroxypropyl cellulose were added immediately before the end of mixing.

Each tablet formulation was compressed at a pre-selected range of forces. An 11/32 inch die with a flat, scored face, bevel edge upper punch and a flat, bevel edge lower punch were selected based on results from a previous study (Rawas-Qalaji *et al.*, 2004). The flat-scored tablets were compressed using a Manesty ® – F3 single-punch tablet press machine (Liverpool, UK).

6.3.3. Evaluation of Tablet Characteristics

Each batch of 200 tablets was collected into a stainless steel beaker. Tablet weight variation, drug content uniformity, and friability were measured using the USP methods and criteria (USP/NF, 2003a, 2003b). Drug content was analyzed using a high performance liquid chromatography system with ultra violet detection (Waters Corp., Milford, MA) and tablet friability was measured using USP Friability instrument (Pharma Test Apparatebau GmbH, Hainburg, Germany). Five tablets were selected randomly from each formulation batch and tested for tablet hardness, disintegration time, and wetting time. The mean ± standard deviation (SD) and percentage of coefficient of variation (CV%) were calculated.

6.3.3.1. Hardness (H)

The H or the crushing tolerance of tablets was measured using an Erweka ® hardness tester (Heusenstamm, Germany).

6.3.3.2. Disintegration Time (DT)

A relatively simple method with rigorous conditions was developed to evaluate the DT of rapidly disintegrating tablets. Each individual tablet was dropped into a 10 ml glass test tube (1.5 cm diameter) containing 2 ml distilled water, and the time required for complete tablet disintegration was observed visually and recorded using a stopwatch. The visual inspection was enhanced by gently rotating the test tube at a 45° angle, without agitation, to distribute any

tablet particles that might mask any remaining undisintegrated portion of the tablets.

6.3.3.3. Wetting Time (WT)

Tablet WT was measured by a procedure modified from that reported by Bi *et al* (1996). The tablet was placed at the center of 2 layers of absorbent paper fitted into a rectangular plastic dish (11 cm X 7.5 cm). After the paper was thoroughly wetted with distilled water, excess water was completely drained out of the dish. The time required for the water to diffuse from the wetted absorbent paper throughout the entire tablet was then recorded by using a stopwatch.

6.3.4. Data Analysis and Curve Fitting

All results were reported as mean ± SD (n=5) and analyzed by plotting H, DT, and WT versus CF; DT and WT versus H and WT versus DT. The relationships were fitted to appropriate equations using Axum 5.0C (MathSoft, Inc.) and NCSS (NCSS, Kaysville, Utah) softwares. The constants of each equation and the correlation of fit (R^2) were calculated using NCSS and Excel 2000 (Microsoft Corporation) softwares.

6.4. Results and Discussion

The powders for all four formulations resulted in good mixing, flowability, and compressibility characteristics. Tablets manufactured from each formulation were within USP specifications for weight variation and drug content uniformity

(USP/NF, 2003b). All formulations passed the USP friability test (USP/NF, 2003a) at the following tablet H values: formulation A ≥ 1.9 ± 0.1 kg, formulation B ≥ 1.8 ± 0.1 kg, formulation C ≥ 2.3 ± 0.2 kg, and formulation D ≥ 2.0 ± 0.2 kg.

6.4.1. Hardness

The H results for increasing CF for each formulation are reported in Table 2 and plotted in Figure 1. A linear increase in the CF resulted in an exponential increase in the tablet H for all four different formulations. Increases in CF possibly reduced the tablet porosity due to a closer rearrangement and compaction of the particles resulting in a harder tablet (Bi et al., 1999; Bi et al., 1996; Marshall, 1986). The exponential increase in the tablet H can be described by Equation 1, where X is CF and Y is H. The equation constants (a and b) for the four formulations are reported in Table 3. Constant b can be used to predict characteristics for tablets prepared with > 24% epinephrine bitartrate. This constant could include factors such as degree of porosity and extent of hydrogen bond formation, but the individual contribution for such factors was not evaluated in this study.

$$Y = ae^{bX} \qquad (1)$$

As epinephrine bitartrate load increased, higher CF was required to maintain the range of H values recorded for formulation A (0% epinephrine bitartrate). This may be due to the poor compressibility of epinephrine bitartrate, which can interfere with, and reduce the formation of, hydrogen bonds between the cellulose particles (Bi et al., 1996). Increasing epinephrine bitartrate loads

result in a greater interference with the interparticle hydrogen bonds formation requiring a higher CF to increase the contact points between the excipient powder particles in order to maintain the desired range of tablet H. Similar results have been reported by Watanabe *et al* (1995), Bi *et al* (1999; 1996), Ishikawa *et al* (2001b), Sugimoto *et al* (2001), and Schiermeier *et al* (2002) for other medications.

6.4.2. Disintegration and Wetting Time

In the USP disintegration test for sublingual tablets, the disintegration apparatus for oral tablets is used without the covering plastic disks (USP/NF, 1990b) and 2 minutes is specified as the acceptable time limit for tablet disintegration (USP/NF, 1990a). The USP apparatus and specifications for the disintegration of sublingual tablets were not suitable for these formulations because the sublingual epinephrine tablets disintegrate so rapidly that differences in DT cannot be measured using them.

An alternative apparatus to detect the differences in oral tablet DT was designed by Bi *et al* (1996). The speed of the apparatus paddle was 100 rpm and the volume of the immersion fluid was 900 ml. These conditions do not reflect the *in vivo* sublingual cavity conditions where a very limited volume (0.35-1.0 ml/min) of saliva is available under normal conditions, with a maximum of 5-7 ml/min after stimulation (Diem *et al.*, 1971). Also, the agitation in the immersion fluid created by the paddle rotation, which would not exist in the sublingual cavity, could enhance tablet disintegration, resulting in a shorter DT compared to what

might be expected in the sublingual cavity. More complicated methods have been used to predict the DT of fast-disintegrating or dissolving tablets by using a texture analyzer (Abdelbary *et al.*, 2005; Dor & Fix, 2000; el-Arini & Clas, 2002).

A relatively simple method, as previously described, was therefore developed to evaluate the DT of these fast-disintegrating sublingual tablets. In this method, the diameter (1.5 cm) of the test tube used is smaller than the diameter of sublingual area in humans (~3-4 cm). The larger sublingual area in humans might actually enhance rather than reduce tablet disintegration. The 1.5-cm diameter of the 10-ml test tube does compare to the sublingual cavity in small laboratory animals such as rabbits, which have been used to date for *in vivo* studies and are being considered for future studies (Rawas-Qalaji et al., 2006). The small volume of water (2 ml) used for tablet disintegration evaluation approximates the volume of saliva secreted under normal conditions. This *in vitro* DT simulates the relatively small sublingual area, the small volume of saliva, and the relatively static environment under the human tongue.

Although a wetting test is not a USP standard test, it is useful for quality control and provides supportive evaluation of these sublingual tablets. Unlike the disintegration test, the wetting test utilizes minimal water, which may be more representative of the quantity of moisture available sublingually. Using this test, the time required for moisture to penetrate the tablet completely is measured and possibly represents the time required to release epinephrine in the presence of minute volumes of saliva. The wetting test designed by Bi *et al* (1996) compares favorably with the conditions in the sublingual area of humans

and animals. This test was modified with regard to the dimensions of the dish, and the volume of water used, as previously described.

The results of the disintegration and wetting tests for each formulation resulting from a range of increasing CF values are reported in Table 2 and plotted in Figures 2 and 3. Formulation A demonstrated an initial linear increase in the DT and WT (Figures 2 and 3) despite the exponential increase in tablet H. When CF was greater than 23.5 KiloNewton (kN), dramatic non-linear increases in DT and WT occurred. Below CF 23.5 kN the linear increase in tablet DT and WT can be described by Equation 2, where X is CF and Y is DT or WT. The equation constants (a and b) for formulation A are recorded in Table 3.

$$Y = bX - a \qquad (2)$$

When the epinephrine bitartrate load was increased for formulations B, C, and D, an exponential increase in the DT and WT resulted from the linear increase in the CF up to 24 kN for formulation B and 25 kN for formulations C and D (Figures 2 and 3). The DT increased dramatically and non-exponentially when CF was greater than 24 kN for formulation B. Formulations C and D resulted in incomplete disintegration and wetting when CF was greater than 25 kN. The exponential increase in tablet DT and WT for formulations B, C, and D can be described by Equation I, where X is CF and Y is DT or WT. The equation constants (a and b) for formulations B, C, and D are reported in Table 3.

Increasing CF probably results in increased particle contact and reduced tablet porosity. The degree of porosity plays an important role in tablet wetting and disintegration. The pores form capillary pathways that allow rapid

water penetration throughout the tablet (Bi et al., 1996; Hedenus *et al.*, 2000; Watanabe et al., 1995). When moistened, the super-disintegrant expands and swells to cause rupture and complete the disintegration of the tablet. The relationship between CF and tablet porosity and its effect on tablet disintegration and wetting have been previously described (Bi et al., 1999; Bi et al., 1996; Schiermeier et al., 2002; Sugimoto et al., 2001; Watanabe et al., 1995).

The degree of bond deformation during compaction also affects tablet disintegration and wetting. Microcrystalline cellulose exhibits both elastic and plastic deformation (Marshall, 1986). Initially, the main type of deformation with increasing CF would be elastic deformation, with particles rearranging to form a compact. When CF exceeds the elastic deformation force, plastic deformation would become the main type of deformation, causing closer and irreversible particle rearrangement. When exposed to small quantities of water, tablets experiencing elastic deformation will demonstrate short DT and WT because the massive expansion of the super-disintegrant will be able to break the bonds formed during compression. Conversely, tablets experiencing plastic deformation will demonstrate longer or incomplete DT and WT. This occurs because the closer particle arrangement possibly results in the formation of numerous, stronger interparticle bonds. In addition, reduced tablet porosity retards water penetration and delays or even inhibits the role of the super-disintegrant at high CF.

In the current study, tablets from all formulations demonstrated initial rapid DT and WT (Figures 2 and 3), despite the initial exponential increase in H

with increasing CF, possibly due to elastic deformation (Figure 1). The dramatic increase in DT and WT (Figures 2 and 3) as the CF exceeds certain critical values probably represents changes from elastic to plastic deformation.

The range of tablet H (Table 2) of formulations C (1.5 ± 0.1 to 6.5 ± 0.2 kg) and D (1.2 ± 0.1 to 4.5 ± 0.1 kg) resulting in complete tablet disintegration and wetting was smaller than for formulations A (1.9 ± 0.1 to 12.0 ± 0.4 kg) and B (1.8 ± 0.1 to 10.3 ± 0.5 kg). Increasing the epinephrine bitartrate load increased tablet H dramatically at higher CF, resulting in longer DT and WT, possibly due to the reduction in the capillary action as a result of lower porosity of the compacted epinephrine bitartrate, and the higher CF required to form a satisfactory tablet compact. At lower CF, the increasing epinephrine bitartrate load in formulations B, C, and D, was less compacted and resulted in shorter DT and WT, comparable to those of formulation A at a given CF (Table 2).

6.4.3. Relationship between Hardness and Disintegration/Wetting Time

The relationship between tablet H and the resulting DT and WT for each formulation are plotted in Figures 4 and 5.

The DT of formulation A was maintained < 10 sec (6.8 ± 0.4 sec) when the tablet H was ≤ 7.2 ± 0.3 kg (Figure 4), despite the exponential increase in tablet H. This small increase in DT as the tablet H was increased in formulation A makes it an ideal candidate to be loaded with increasing doses of epinephrine bitartrate.

Loading formulation A with increasing epinephrine bitartrate loads in formulations B, C, and D resulted in lower tablet H and shorter DT at a given CF values (Table 2). The DT was maintained below 10 sec at tablet H for formulations B ≤ 4.9 ± 0.6 kg, C ≤ 4.0 ± 0.3 kg, and D ≤ 3.1 ± 0.2 kg (Table 2). Further increases in tablet H up to 6.5 ± 0.2 kg for formulation C and 4.5 ± 0.1 kg for formulations D, still resulted in short DT values of 14.0 ± 1.4 sec for formulation C and 26.0 ± 6.4 sec for formulation D. Formulations B, C, and D retained short tablet DTs (Figure 4) without compromising tablet H and friability. Based on the USP friability criteria (USP/NF, 2003a), these tablet formulations can withstand shipping and handling when tablet H is maintained at least ≥ 2.3 ± 0.2 kg.

Similar results were obtained by plotting tablet WT against H for each formulation. The WT of formulation A was maintained < 30 sec, despite the exponential increase in the tablet H up to 7.2 ± 0.3 kg (Figure 5). In contrast, with increasing epinephrine bitartrate loads for the other formulations, a rapid WT (< 30 sec) required that tablet H be maintained for formulations B ≤ 4.9 ± 0.6 kg, C ≤ 4.0 ± 0.3 kg, and D ≤ 3.1 ± 0.2 kg (Table 2).

The correlation between the DT and WT of different formulations results in a linear relationship between DT and WT (Figure 6), as previously reported by Bi *et al* (1996) and Aly *et al* (2005), where the degree of tablet porosity appears to be the common factor. The data fitted to Equation II supports this correlation (where X is DT and Y is WT), and the equation constants (a and b) for the four formulations are reported in Table 3.

6.5. Conclusions

Tablets with drug loads from 0% to 24% epinephrine can be formulated with hardness, disintegration times, and wetting times suitable for sublingual administration and might be potentially useful for the emergency treatment of anaphylaxis. The sublingual bioavailability of epinephrine from formulations B, C, and D are being evaluated in a validated rabbit model.

6.6. Unpublished Amendment

During the dose ranging studies in a rabbit model, a tablet formulation containing 48% epinephrine bitartrate, equivalent to 40 mg epinephrine, was required to achieve plasma epinephrine concentrations similar to those obtained after epinephrine 0.30 mg intramuscular injection in the thigh.

Data from these increasing drug load studies were used to obtain the required parameters to manufacture fast-disintegrating tablets loaded with 48% epinephrine bitartrate. These 40 mg epinephrine tablets resulted in the following characteristics: mean H (± SD) 2.4 ± 0.3 kg with CV% of 12.4%, DT 13.5 ± 0.6 sec with CV% of 4.1%, WT 26.2 ± 4.5 sec with CV% of 17.0%, and friability 0.6%.

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6.8. Tables

Table 1. Composition of the 4 tablet formulations of epinephrine*

	Tablet Formulations			
Ingredient Weight %	A	B	C	D
Epinephrine Bitartrate	-	6	12	24
Microcrystalline Cellulose (PH-301)	88.2	82.8	77.4	66.6
Low-Substituted Hydroxypropyl Cellulose (LH11)	9.8	9.2	8.6	7.4
Magnesium Stearate	2	2	2	2

*Tablet weight was 150 mg.

Table 2. The Effect of increasing compression force on the tablet hardness, disintegration time, and wetting time*

	A						B						C						D					
CF	H	CV	DT	CV	WT	CV	H	CV	DT	CV	WT	CV	H	CV	DT	CV	WT	CV	H	CV	DT	CV	WT	CV
21.5	1.9	4.5	2.2	20.3	8.2	5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22.0	2.5	6.6	3.2	14.0	11.4	7.8	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
22.5	3.6	5.9	5.2	8.6	13.4	17.2	1.8	4.7	2.8	16.0	7.2	6.2	-	-	-	-	-	-	-	-	-	-	-	-
23.0	4.7	8.5	6.8	21.8	14.0	14.3	2.5	7.3	3.8	11.8	8.8	9.5	-	-	-	-	-	-	-	-	-	-	-	-
23.5	7.2	4.5	8.0	8.8	15.8	13.7	4.1	5.1	6.2	13.5	11.0	6.4	1.5	5.5	4.6	11.9	8.6	10.3	1.2	9.8	4.6	11.9	9.0	7.9
24.0	12.0	3.0	37.2	5.8	86.0	18.7	4.9	11.4	9.0	11.1	20.8	10.4	2.3	10	5.8	7.7	16.6	10.9	2.0	8.1	5.6	9.8	16.4	7.0
24.5	-	-	-	-	-	-	10.3	4.6	120.0	6.6	102.4	21.1	4.0	7.7	7.6	11.8	24.4	6.9	3.1	6.6	9.4	9.5	27.2	12.6
25.0	-	-	-	-	-	-	-	-	-	-	-	-	6.5	3.4	14.0	10.1	75.6	15.8	4.5	2.9	26.0	24.8	83.6	38.0
25.5	-	-	-	-	-	-	-	-	-	-	-	-	9.0	12.9	>120	-	>120	-	9.1	1.4	>120	-	>120	-

*CF indicates compression force (kN); H, tablet hardness (kg); CV, coefficient of variation (%); DT, disintegration time (sec); WT, wetting time (sec).

Table 3. Correlation constants, a and b, for the 4 tablet formulations*

	A		B		C		D	
Constants for	a	b	a	b	a	b	a	b
H vs. CF	$3x10^{-07}$	0.72	$1x10^{-08}$	0.83	$7x10^{-10}$	0.92	$1x10^{-10}$	0.98
DT vs. CF	63.32†	3.04†	$4x10^{-08}$	0.80	$2x10^{-07}$	0.72	$8x10^{-12}$	1.14
WT vs. CF	67.54†	3.56†	$1x10^{-6}$	0.68	$6x10^{-14}$	1.38	$2x10^{-14}$	1.44
WT vs. DT †	-1.26	2.26	2.44	2.70	26.25	7.19	4.71	3.40

*CF indicates compression force (kN); H, tablet hardness (kg); CV, coefficient of variation; DT, disintegration time (sec); WT, wetting time (sec).
†Constants derived using equation 2 (all other constants derived using equation 1).

6.9. Figures

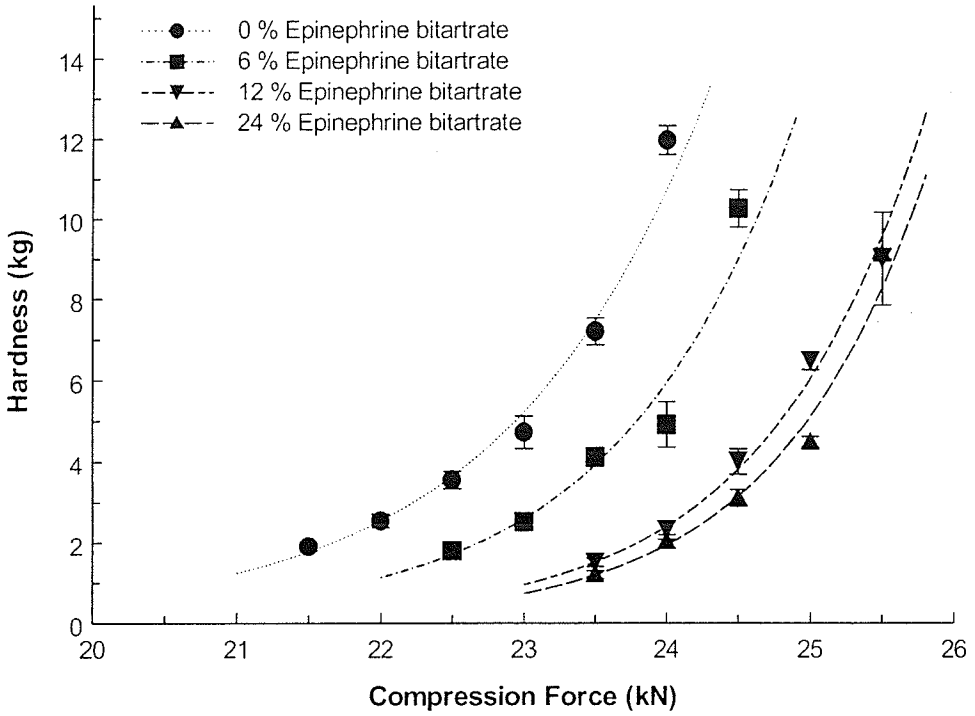

Figure 1: Effect of increasing compression force on tablet hardness of 0%, 6%, 12%, and 24% epinephrine bitartrate tablet formulations. Data are represented as mean ± SD (n = 5). R^2 is ≥ 0.97 in all formulations.

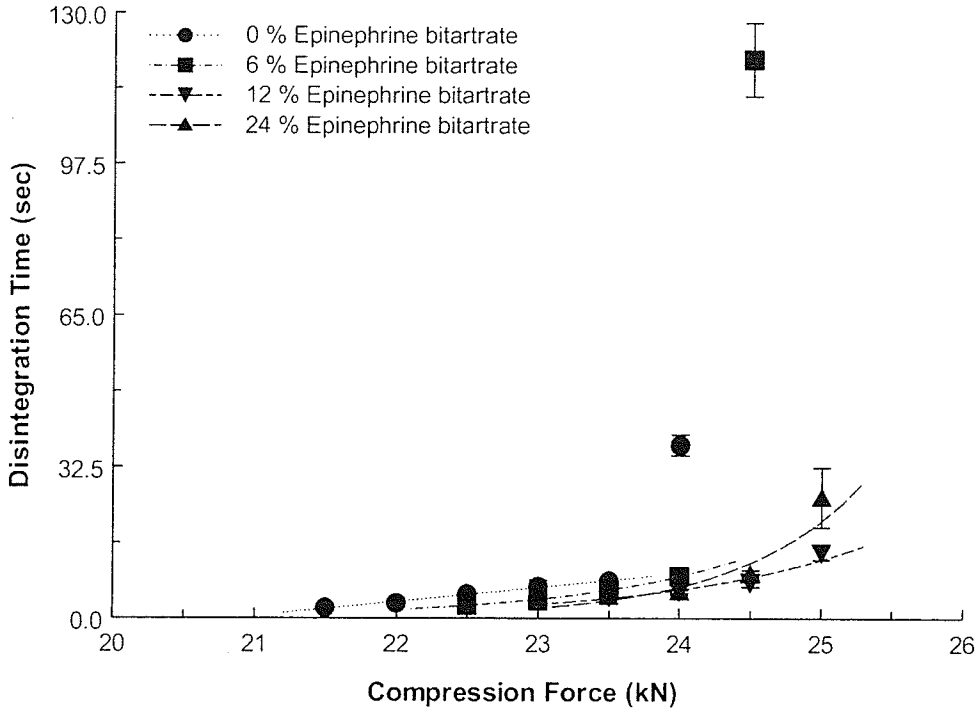

Figure 2: Effect of increasing compression force on tablet disintegration time of 0%, 6%, 12%, and 24% epinephrine bitartrate tablet formulations. Data are represented as mean ± SD (n = 5). R^2 is ≥ 0.91 in all formulations.

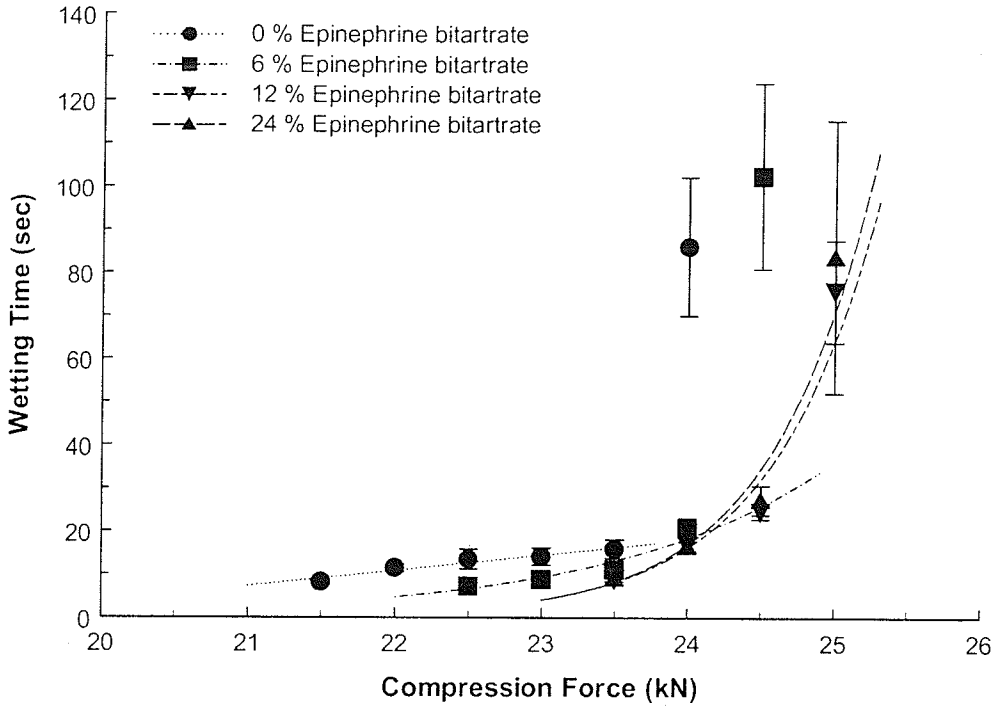

Figure 3: Effect of increasing compression force on tablet wetting time of 0%, 6%, 12%, and 24% epinephrine bitartrate tablet formulations. Data are represented as mean ± SD (n = 5). R^2 is ≥ 0.91 in all formulations.

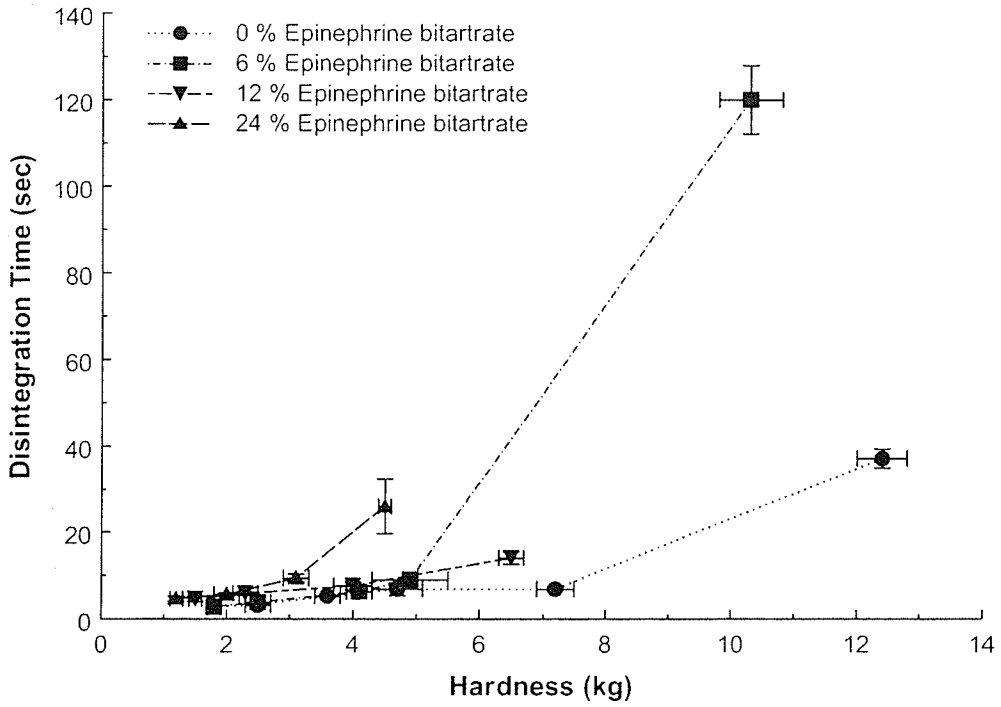

Figure 4: Relationship between tablet hardness and disintegration time of 0%, 6%, 12%, and 24% epinephrine bitartrate tablet formulations. Data are represented as mean ± SD (n = 5).

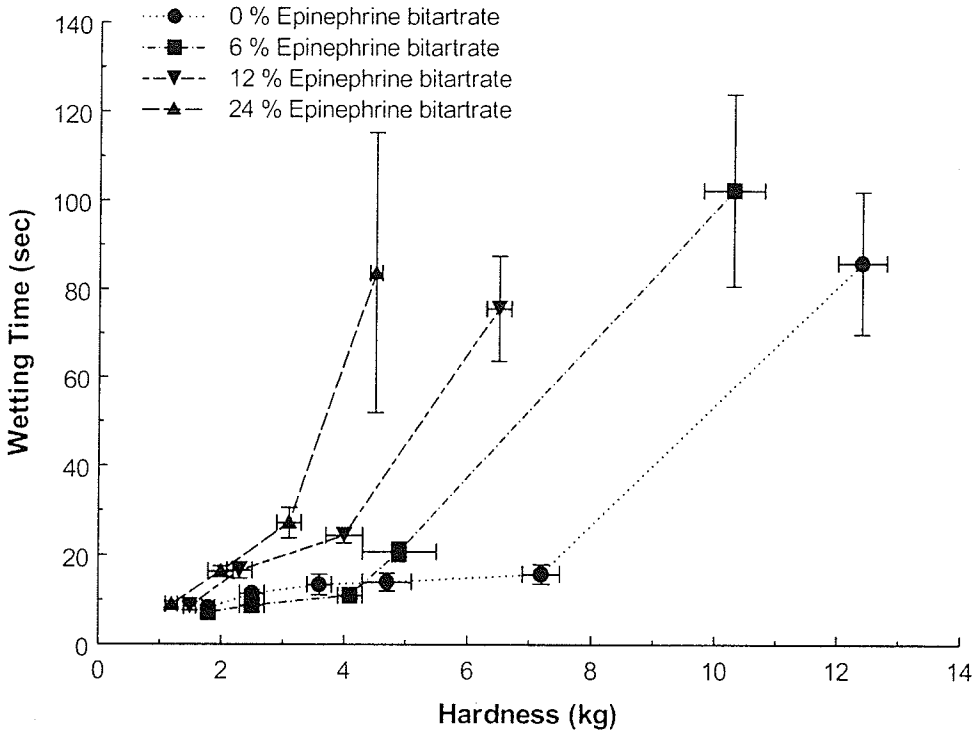

Figure 5: Relationship between tablet hardness and wetting time of 0%, 6%, 12%, and 24% epinephrine bitartrate tablet formulations. Data are represented as mean ± SD (n = 5).

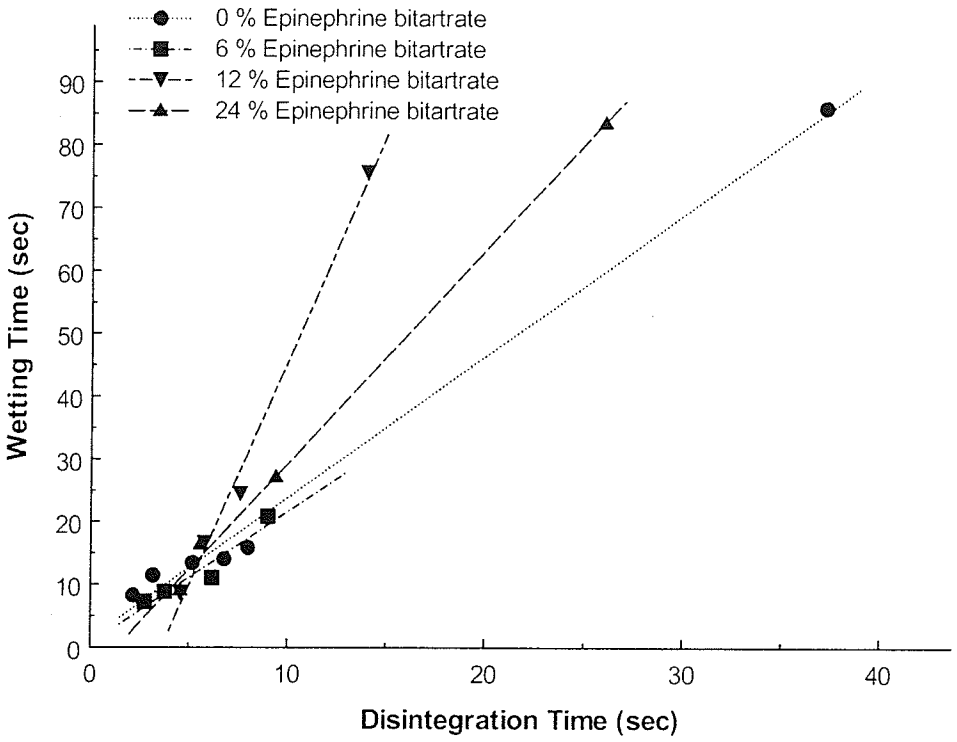

Figure 6: Correlation between tablet disintegration time and wetting time of 0% 6%, 12%, and 24% epinephrine bitartrate tablet formulations. Data are represented as mean ± SD (n = 5). R^2 is ≥ 0.98 in all formulations.

CHAPTER VII: Fast-Disintegrating Sublingual Epinephrine Tablets: Long Term Stability Study

7.1. Abstract

Purpose: To assess the stability of epinephrine (E) in a novel fast-disintegrating sublingual tablet formulation for the potential first-aid emergency treatment of anaphylaxis.

Methods: Three tablet batches, containing 10 mg (E-10), 20 mg (E-20), and 40 mg (E-40) of E, were manufactured by direct compression, and tested for tablet weight variation and content uniformity. Tablets were stored in tightly closed, opaque, plastic containers with desiccants at 25°C, 5°C, and 5°C under nitrogen (5°C-N_2). Results from tablet content uniformity tests were used as the controls. From each batch, 6 tablets were randomly selected and removed from each storage condition at 6 months and 12 months for E-10 and E-20 and at 20 months for E-40. Tablets were inspected visually for color change, and analyzed for E content using HPLC-UV. E dose remaining (EDR) was calculated for each tablet and analyzed statistically ($p<0.05$).

Results: Slight yellow discoloration was observed only for E-40 mg tablets stored at 25°C for 20 months. Mean (±SEM) EDR in E-10 and in E-20 tablets stored for 12 months at 25°C, 9.6±0.1 and 19.4±0.4 mg respectively, 5°C, 9.7±0.2 and 20.3±0.3 mg respectively, and 5°C-N_2, 9.6±0.1 and 20.9±0.8 mg respectively, did not differ significantly from the controls, 9.8±0.1 and 20.1±0.3

mg respectively, and from each other. EDR in E-40 tablets stored for 20 months at 25°C, 37.5±0.2 mg, 5°C, 38.9±0.6 mg, and 5°C-N_2, 38.5±1.2 mg, did not differ significantly from the control, 38.0±0.6 mg, and from each other.

Conclusion: These E tablets are stable and have the potential for the emergency treatment of anaphylaxis.

7.2. Introduction

For the first-aid emergency treatment of anaphylaxis, epinephrine is the drug of choice. It is available in injectable dosage forms (Chamberlain, 1999; Ellis & Day, 2003; Lieberman, 2003; Sampson *et al.*, 2006; F. E. R. Simons, 2004). Epinephrine auto-injectors such as EpiPen®, EpiPen Jr® (Dey LP, Nappa, CA), Twinjet 0.3 mg®, and Twinjet 0.15® (Verus Pharmaceuticals, Inc. San Diego, CA) are widely prescribed for out-of-hospital emergency treatment of anaphylaxis. However, self-injectable epinephrine is underutilized when anaphylaxis occurs (Bock *et al.*, 2001; Gold & Sainsbury, 2000) due to several drawbacks (Gold & Sainsbury, 2000; Korenblat *et al.*, 1999; Sicherer *et al.*, 2000; F. E. R. Simons, 2004, 2005).

Epinephrine is extensively metabolized after oral administration by the catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver (Lefkowitz *et al.*, 1996). In aqueous solutions, epinephrine is unstable in the presence of oxygen, light, heat, and neutral or alkaline pH values (Connors *et al.*, 1986). As a powder,

epinephrine should be stored in a cool, dry place and in a light-resistant container (USP/NF, 1990).

The sublingual route of administration is a promising alternative route for epinephrine administration. Drugs that can be absorbed sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Bredenberg *et al.*, 2003; Cunningham *et al.*, 1994; Glover *et al.*, 2002; Guez, 2003; Kroboth *et al.*, 1995; Price *et al.*, 1997; Saxena *et al.*, 2005). The high vascularity of the sublingual mucosa and the low molecular weight of the lipophilic epinephrine facilitate rapid absorption directly into the venous circulation through the sublingual and frenular veins.

The sublingual absorption of epinephrine has been documented in a validated rabbit model (Rawas-Qalaji *et al.*, 2006a) using fast-disintegrating tablet formulations, and in humans (K. J. Simons *et al.*, 2004) using epinephrine powder.

Fast-disintegrating tablets for sublingual administration, which retain sufficient hardness to withstand shipping and handling, have been formulated using a direct compression method at a range of epinephrine doses (Rawas-Qalaji *et al.*, 2006b). These tablets have been shown to release epinephrine rapidly (Rawas-Qalaji *et al.*, 2006b) and the epinephrine absorbed following the sublingual administration would have the potential for the emergency treatment of anaphylaxis. The stability of these epinephrine tablets during manufacturing processes and after storage has not been evaluated previously.

The aim of this study was to evaluate the stability of epinephrine in these fast-disintegrating tablets under a series of conditions at which tablets may be commonly stored.

7.3. Materials and Methods

7.3.1. Materials

(-)-Epinephrine (+) bitartrate, (-)-3,4-dihydroxy-α-[(methylamino)methyl]benzyl alcohol (+)-tartrate (1:1) salt, was purchased from Sigma-Aldrich (St. Louis, MO). Ceolus ® PH-301 (microcrystalline cellulose) with a mean particle size of 50 μm was supplied by Asahi Kasei Chemicals Corp (Tokyo, Japan) and low-substituted hydroxypropyl cellulose (LH11) with a mean particle size of 50 μm was supplied by Shin-Etsu Chemical Co (Tokyo, Japan). Magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ).

7.3.2. Preparation and Evaluation of Tablets

Three different batches of fast-disintegrating tablet containing 10 mg, 20 mg, and 40 mg of epinephrine were manufactured by direct compression. These tablets were formulated using microcrystalline cellulose, low-substituted hydroxylpropyl cellulose, and magnesium stearate as described in our previous study (Rawas-Qalaji *et al.*, 2006b). The tablet weight was 150 mg. All excipients were kept under low humidity condition before mixing. The mixing process was performed in a light-resistant container after flushing the container with nitrogen. The prepared powder mixture of the three tablet batches were compressed

directly after mixing at a pre-selected compression force for each tablet batch that permits rapid tablet disintegration and wetting, while retaining sufficient hardness to withstand shipping and handling based on results from our previous study (Rawas-Qalaji *et al.*, 2006b). All batches were tested for tablet weight variation and drug content uniformity using the USP methods and criteria (USP/NF, 2003). Each tablet was dissolved in 2.0 mL solvent of 0.1 M perchloric acid and 0.1 mM sodium metabisulfite (Hjemdahl, 1987). Aliquots of 50 μL were withdrawn and diluted to 2.0 mL with the solvent. Drug content was analyzed using high performance liquid chromatography system with ultra-violet detection (Waters Corp., Milford, MA).

7.3.3. Storage of Tablets

Each of the three tablet batches was divided into three equal portions and immediately transferred into tightly closed, opaque, plastic tablet containers with desiccants. Container 1 was stored at 25 °C (room temperature), container 2 was stored at 5 °C (refrigerator), and container 3 was flushed with nitrogen before being tightly closed and stored at 5 °C. From the three containers of the 10 mg and 20 mg epinephrine tablet batches six-tablet samples, randomly selected, were withdrawn at six and twelve months. Containers stored under nitrogen were re-flushed with nitrogen before being sealed and stored for the next time period. From the three containers of the 40 mg epinephrine tablet batch six-tablet samples, randomly selected, were withdrawn at twenty months.

The six tablets in each sample were observed immediately for any obvious visual changes, and then dissolved and diluted to be analyzed for epinephrine content using a reverse phase high performance liquid chromatography system (Waters Corp., Milford, MA) with ultra violet detection (USP/NF, 1990). The mean ± standard error (SEM) epinephrine dose data obtained from the content uniformity test for each tablet batch were used as the control epinephrine content values at baseline before storage commenced.

7.3.4. Data Analysis

For each tablet batch, the epinephrine dose remaining in the tablets selected from the three containers, stored under the three different storage conditions, for different storage periods were calculated and compared with each other and with control using two-way ANOVA and Tukey-Kramer tests using NCSS Statistical Analysis Software (NCSS, Kaysville, UT). Differences were considered to be significant at $p < 0.05$.

7.4. Results

All three tablet batches were within USP specifications for weight variation and drug content uniformity (USP/NF, 2003).

There were no detectable color changes in the 10 mg and 20 mg epinephrine tablet batches stored for six and twelve months under the three storage conditions. Also, there were no detectable color changes in the 40 mg epinephrine tablet batch stored for twenty months at 5 °C with and without

nitrogen flushing prior to storage. Slight tablet discoloration was observed in the 40 mg epinephrine tablet batch stored for twenty months at 25 °C.

Mean (± SEM) epinephrine doses remaining in the 10 mg and 20 mg epinephrine tablet batches stored for six and twelve months, and in 40 mg epinephrine tablet batch stored for twenty months at 25 °C, 5 °C, and 5 °C with nitrogen flushing are reported in Table 1.

For the 10 mg epinephrine tablet batch, the mean (± SEM) epinephrine dose remaining in tablets stored for six months at 25 °C, 9.2 ± 0.1 mg, 5 °C, 9.3 ± 0.2 mg, and at 5 °C with nitrogen flushing, 9.4 ± 0.3 mg, and for twelve months at 25 °C, 9.6 ± 0.1 mg, 5 °C, 9.7 ± 0.2 mg, and at 5 °C with nitrogen flushing, 9.6 ± 0.1 mg, did not differ significantly from each other and from the control, 9.8 ± 0.1 mg.

For the 20 mg epinephrine tablet batch, the mean (± SEM) epinephrine dose remaining in tablets stored for six months at 25 °C, 19.8 ± 0.5 mg, 5 °C, 19.8 ± 0.5 mg, and at 5 °C with nitrogen flushing, 20.3 ± 0.3 mg, and for twelve months at 25 °C, 19.4 ± 0.4 mg, 5 °C, 20.3 ± 0.3 mg, and at 5 °C with nitrogen flushing, 20.9 ± 0.8 mg, did not differ significantly from each other and from the control, 20.1 ± 0.3 mg.

For the 40 mg epinephrine tablet batch, the mean (± SEM) epinephrine dose remaining in tablets stored for twenty months at 25 °C, 37.5 ± 0.2 mg, 5 °C, 38.9 ± 0.6 mg, and at 5 °C with nitrogen flushing, 38.5 ± 1.2 mg, did not differ significantly from each other and from the control, 38.0 ± 0.6 mg.

7.5. Discussion

The stability of epinephrine in solutions has been thoroughly investigated, and the optimal pH, storage conditions, and the quantities of antioxidants required to stabilize epinephrine have been determined (Connors *et al.*, 1986). The recommendations for the storage of epinephrine as a powder are documented in the USP and stated by the manufacturers. However, there are no data about the stability of epinephrine in a tablet dosage form and the effect of tablet manufacturing processes on the stability of epinephrine.

In this study, the results from the USP content uniformity test for the three epinephrine tablet batches suggest that the manufacturing procedures and processes used here do not affect the stability of epinephrine. The direct compression method is commonly used for heat and moisture sensitive active ingredients (Sheth *et al.*, 1980). Also, using light-resistant containers flushed with nitrogen for mixing, maintaining the excipients under low humidity before mixing, and compressing the powder mixture directly after mixing may have played a role in minimizing any oxidative reactions during manufacturing.

These fast-disintegrating epinephrine tablets were stable for twelve months under the three storage conditions, 25 °C, 5 °C, and 5 °C with nitrogen flushing. They were stable for twenty months at 5 °C with and without nitrogen flushing. The epinephrine dose remaining in the 40 mg epinephrine tablets stored for twenty months at 25 °C did not differ significantly from control and from tablets stored at 5 °C with and without nitrogen flushing. However, the slight discoloration of the tablets requires further investigation based on the USP

standards for epinephrine injections (USP/NF, 1990). These results showed that the use of opaque containers to reduce light, desiccants to reduce humidity in the container, and low temperatures prevented tablet discoloration for at least twenty months. Exposing the tablets to oxygen at 25 °C, 5 °C did not affect the stability of epinephrine since flushing the container with nitrogen prior storage at 5 °C did not result in significantly higher epinephrine content in these tablets.

Classical measures were used to maintain the stability of epinephrine in these tablets. It has been reported that the hydroperoxide content in some of the commonly used excipients in pharmaceutical formulations may contribute to oxidative reactions in labile medications. Temperature increase was also found to increase the hydroperoxide content in these excipients. However, microcrystalline cellulose, the main excipient used for the formulation of these epinephrine tablets, was reported to contain only minute levels of hydroperoxide (< 10 nmole/g) and these levels did not increase after being exposed to elevated temperatures for four weeks (Wasylaschuk *et al.*, 2005).

7.6. Conclusion

Fast-disintegrating sublingual tablets containing a range of epinephrine doses, 10, 20 and 40 mg, are stable and could be useful for the potential emergency treatment of anaphylaxis. The sublingual bioavailability of epinephrine from these fast-disintegrating sublingual tablets has been evaluated in the validated rabbit model (Rawas-Qalaji *et al.*, 2006a).

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Table 1. Epinephrine doses remaining in 10 mg, 20 mg, and 40 mg epinephrine tablet batches stored at 25 °C, 5 °C, and 5 °C with nitrogen flushing (5 °C-N_2) for six, twelve, and twenty months[a]

	10 mg epinephrine tablets[b]		20 mg epinephrine tablets[c]		40 mg epinephrine tablets[d]
Storage condition	6 Months	12 Months	6 Months	12 Months	20 Months
25 °C	9.2 ± 0.1	9.6 ± 0.1	19.8 ± 0.5	19.4 ± 0.4	37.5 ± 0.2
5 °C	9.3 ± 0.2	9.7 ± 0.2	19.8 ± 0.5	20.3 ± 0.3	38.9 ± 0.6
5 °C-N_2	9.4 ± 0.3	9.6 ± 0.1	20.3 ± 0.3	20.9 ± 0.8	38.5 ± 1.2

[a]mean ± SEM (n = 6).

[b]epinephrine dose in the control tables was 9.8 ± 0.1 mg.

[c]epinephrine dose in the control tablets was 20.1 ± 0.3 mg.

[d]epinephrine dose in the control tables was 38.0 ± 0.6 mg.

CHAPTER VIII: Sublingual Epinephrine Tablets Versus Intramuscular Injection of Epinephrine: Dose Equivalence for Potential Treatment of Anaphylaxis*

8.1. Abstract

Background: Epinephrine auto-injectors are underutilized in the emergency treatment of anaphylaxis in the community, perhaps in part because of fear of needles.

Objectives: To determine the sublingual epinephrine dose from a novel fast-disintegrating tablet required to achieve epinephrine plasma concentrations (EPCs) similar to those obtained after epinephrine 0.3 mg intramuscularly injection.

Methods: In a prospective 5-way crossover study, sublingual tablets containing epinephrine 0, 10, 20, and 40 mg, and epinephrine 0.3 mg intramuscularly in the thigh (EpiPen®) were compared in a validated rabbit model. Blood samples were collected before dosing and 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, and 180 min afterwards. EPCs were measured using HPLC-EC. Pharmacokinetic parameters were calculated using WinNonlin®.

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Results: The area under the curve (AUC), maximum concentration (C_{max}), and time at which C_{max} was achieved (T_{max}) did not differ significantly ($p>0.05$) after epinephrine 40 mg (AUC=1,861±537 ng/ml/min, C_{max}=31.0±13.1 ng/ml, and T_{max}=9±2 min) and epinephrine 0.3 mg intramuscular (AUC=2,431±386 ng/ml/min, C_{max}=50.3±17.1 ng/ml, and T_{max}=21±5 min). The AUC after tablets containing epinephrine 0 mg (AUC=472±126 ng/ml/min), epinephrine 10 mg (AUC=335±152 ng/ml/min), and epinephrine 20 mg (AUC=801±160 ng/ml/min) did not differ significantly from each other, but were significantly lower ($p<0.05$) than the AUC after epinephrine 0.3 mg intramuscularly.

Conclusion: Sublingual administration of epinephrine 40 mg from this tablet formulation resulted in EPCs similar to those obtained after epinephrine 0.3 mg intramuscular injection in the thigh.

Clinical Implication: For treatment of anaphylaxis in the community, self-injectable epinephrine is underutilized. This novel, fast-disintegrating epinephrine tablet formulation for sublingual administration is a feasible alternative that warrants further development.

8.2. Introduction

There is universal agreement that prompt epinephrine injection is the drug of choice for the treatment of anaphylaxis (Lieberman, 2003; McLean-Tooke *et al.*, 2003; Sampson *et al.*, 2006; Simons, 2004). The recommended epinephrine dose for the treatment of anaphylaxis is 0.3-0.5 mg in adults and 0.01 mg/kg, up to a maximum of 0.3 mg, in children, given by intramuscular injection (Lieberman,

2003; McLean-Tooke *et al.*, 2003; Sampson *et al.*, 2006; Simons, 2004). These recommendations are based on clinical experience and/or studies in healthy volunteers (Simons *et al.*, 2001b), rather than on randomized, double-blind, placebo-controlled dose-ranging studies in patients experiencing anaphylaxis, which are impossible to perform from the ethical standpoint (Simons, 2004).

Most anaphylactic reactions occur unexpectedly in the community due to foods, insect stings, medications, natural rubber latex, and other triggers (Lieberman, 2003; Sampson *et al.*, 2006; Simons, 2004). For out-of-hospital emergency treatment of anaphylaxis, epinephrine auto-injectors such as EpiPen®, EpiPen Jr® (Dey LP, Nappa, CA), Twinject 0.3 mg®, and Twinject 0.15 mg® (Verus Pharmaceuticals, Inc. San Diego, CA) are prescribed, however, self-injectable epinephrine is underutilized when anaphylaxis occurs (Bock *et al.*, 2001; Gold & Sainsbury, 2000). The drawbacks of epinephrine auto-injectors include: high cost which limits affordability and availability worldwide (Simons, 2005); perceived large size and bulkiness; limitations on repeat dosing (if required) (Korenblat *et al.*, 1999); fear and anxiety associated with the use of needles (Simons, 2004); and dosing errors due to incorrect technique of administration (Gold & Sainsbury, 2000; Sicherer *et al.*, 2000). In addition, it is impossible to give an accurate dose to infants and to many children using currently available auto-injectors, which provide only two different premeasured, fixed epinephrine doses, 0.15 mg and 0.3 mg (Simons, 2004). Alternatives to an epinephrine autoinjector, such as an epinephrine ampule/syringe/needle or an

epinephrine metered dose inhaler are impractical with regard to rapid and accurate dosing (Simons, 2004; Simons *et al.*, 2001a; Simons *et al.*, 2000a).

The sublingual route of administration is a promising alternative route for epinephrine administration. Drugs that are absorbed sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Motwani *et al.*, 1991; Price *et al.*, 1997). Lipophilic drugs with a low molecular weight such as epinephrine are likely absorbed across the sublingual mucosa into the venous circulation by transcellular diffusion (Birudaraj *et al.*, 2005), a mechanism driven by the concentration gradient (Figure 1) (Sherwood, 2004).

The aims of this study were to confirm the feasibility of epinephrine absorption from a novel sublingual tablet formulation, to ascertain if absorption was dose-dependent, and to determine the sublingual dose required to achieve plasma epinephrine concentrations similar to those obtained after the intramuscular injection of 0.3 mg epinephrine.

8.3. Methods

The research was conducted according to current guidelines published by the Canadian Council on Animal Care (Olfert *et al.*, 1993) and was approved by the University of Manitoba Protocol Management and Review Committee.

Novel rapidly disintegrating tablets containing 0 mg (placebo), 10 mg, 20 mg, and 40 mg epinephrine were formulated in the manufacturing laboratory of

the Faculty of Pharmacy at the University of Manitoba. They contained no lactose, and met USP standards for tablet weight variation, content uniformity, and friability. They disintegrated in less than 15 sec, as assessed using a novel *in vitro* disintegration test developed to simulate the sublingual environment (Rawas-Qalaji *et al.*, 2004a; Rawas-Qalaji *et al.*, 2004b).

In a prospective, controlled, 5-way crossover study, five New Zealand white rabbits (mean weight 4.8 ± 0.2 Kg) were investigated on five different study days at least four weeks apart, using a protocol described previously (Gu *et al.*, 1999). Each rabbit received epinephrine 10 mg, 20 mg, and 40 mg as sublingual tablets, a placebo sublingual tablet (epinephrine 0 mg) as a negative control, and epinephrine 0.3 mg by intramuscular injection in the right thigh from an EpiPen® as a positive control.

For sublingual epinephrine tablet administration, the rabbit's mouth was opened and a wooden rod was inserted between the jaws. The tongue was elevated using flat forceps, and the tablet was placed underneath using another pair of forceps. The mouth was gently, but firmly held shut for 5 minutes with the wooden rod in place to prevent chewing or swallowing the tablet. 0.3-0.5 mL water was administered immediately after dosing to facilitate tablet disintegration. An additional 0.7-0.5 mL of water was administered at the end of the 5 minute immobilization time, in order to remove any remaining epinephrine from under the tongue.

Epinephrine 0.3 mg was injected intramuscularly in the thigh using an EpiPen® after which the solution remaining in the EpiPen® was evacuated into a

polystyrene test tube and frozen at –20 °C, to be analysed for epinephrine content using a reverse phase high performance liquid chromatography (HPLC) system (Waters Corp., Milford, MA.) with ultra violet detection (UV) (USP/NF, 1990).

8.3.1. Measurement of Plasma Epinephrine Concentrations

An indwelling catheter (OPTIVA® 22G 1", Johnson & Johnson Medical, Arlington, TX) was inserted into an ear artery 30 min before dosing. A 2 mL blood sample was obtained immediately before dosing and at 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, and 180 minutes afterwards.

Blood samples were refrigerated within 1 hour of sampling and centrifuged at 4 °C. Plasma was frozen at –20 °C. Before analysis, plasma was thawed at room temperature and epinephrine was extracted by a solid-liquid extraction process, with an efficiency of 84%. Epinephrine concentrations were measured using HPLC system (Waters Corp., Milford, MA.) with electrochemical detection (EC) (Ganhao *et al.*, 1991; Hjemdahl, 1984, 1987). Two calibration curves with two different epinephrine concentration ranges were prepared. The low range calibration curve was linear over the range of 0.1 to 1.0 ng/mL with a coefficient of variation of 0.8 % at 0.1 ng/mL and 1.5% at 1.0 ng/mL. The high range calibration curve was linear over the range of 1.0 to 10.0 ng/mL with a coefficient of variation of 5.0% at 1.0 ng/mL and 1.2% at 10.0 ng/mL.

8.3.2. Data Analysis

Mean (±SEM) maximum plasma epinephrine concentrations (C_{max}), the times at which C_{max} were achieved (T_{max}), and the area under the plasma concentration versus time curves (AUC) were calculated by a trapezoidal rule from the plasma epinephrine concentration versus time plot of each individual rabbit using WinNonlin® 5.0 (Pharsight, Mountain View, CA). The AUC, C_{max}, and T_{max} values for each rabbit were compared using repeated measures ANOVA, Tukey-Kramer tests, and paired Students' t-test using NCSS Statistical Analysis Software (NCSS, Kaysville, UT). Differences were considered to be significant at $p < 0.05$.

8.4. Results

The mean (±SEM) epinephrine dose injected using EpiPen® auto-injectors was 0.34 ± 0.002 mg as calculated by multiplying the epinephrine concentration measured in the solution remaining in the EpiPens® after injection by the stated injected volume (0.3 mL).

Mean (±SEM) plasma epinephrine concentration versus time plots after the administration of epinephrine 0 mg (placebo), epinephrine 10 mg, epinephrine 20 mg and epinephrine 40 mg sublingual tablets, and epinephrine 0.3 mg intramuscularly are shown in Figure 2 and Figure 3. Mean (±SEM) AUC, $C_{baseline}$ (endogenous), C_{max}, and T_{max} values, after the administration of placebo, epinephrine 10 mg, epinphrine 20 mg and epinephrine 40 mg sublingual tablets, and epinephrine 0.3 mg intramuscularly are shown in Table 1.

Mean (±SEM) AUC after the administration of epinephrine 40 mg sublingual tablets (1861 ± 537 ng/ml/min) and epinephrine 0.3 mg intramuscularly (2431 ± 386 ng/ml/min) did not differ significantly. Mean AUC after the administration of epinephrine 10 mg (335 ± 152 ng/ml/min) and epinephrine 20 mg (801 ± 160 ng/ml/min) sublingual tablets were significantly lower than after epinephrine 0.3 mg intramuscularly (2431 ± 386 ng/ml/min), and not significantly higher than after placebo sublingual tablets (472 ± 126 ng/ml/min). The bioavailability of sublingual epinephrine, as assessed using AUC, increased linearly with a linear increase in dose (Figure 4).

Mean (±SEM) C_{max} values after epinephrine 40 mg sublingual tablets (31.0 ± 13.1 ng/ml) and epinephrine 0.3 mg intramuscularly (50.3 ± 17.1 ng/ml) did not differ significantly. Mean C_{max} values after epinephrine 10 mg (5.2 ± 2.3 ng/ml) and epinephrine 20 mg (6.6 ± 1.4 ng/ml) sublingual tablets were significantly lower than after epinephrine 0.3 mg intramuscularly (50.3 ± 17.1 ng/ml), and not significantly higher than after placebo sublingual tablets (6.5 ± 1.3 ng/ml).

Mean (±SEM) T_{max} after the administration of epinephrine 10 mg (37 ± 11 min), epinephrine 20 mg (31 ± 9 min) and epinephrine 40 mg (9 ± 2 min) sublingual tablets, and epinephrine 0.3 mg intramuscularly (21 ± 5 min) did not differ significantly.

No adverse effects were observed.

8.5. Discussion

The readily accessible, convenient sublingual route of administration has long been used to administer medications such as nitroglycerine. The high vascularity of the sublingual mucosa facilitates rapid drug absorption directly into the venous circulation through the sublingual and frenular veins, bypassing the gastrointestinal tract, the hepatic portal circulation, and hepatic first-pass metabolism (Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Motwani *et al.*, 1991; Price *et al.*, 1997). Further drug absorption can be terminated if necessary by removing the tablet from the sublingual space. Sublingual epinephrine tablets should be less expensive to produce than the currently available auto-injectors are. They are unobtrusive to carry and to self-administer and repeat dosing is practical. They can be formulated in a range of epinephrine doses to provide accurate dosing for individuals with a wide range of body weights.

The most important rationale for using a rabbit model for epinephrine pharmacokinetic studies was that this epinephrine-tolerant species does not develop any apparent adverse effects after high doses of epinephrine on a mg/kg basis relative to human doses; epinephrine 0.3 mg intramuscularly from an auto-injector was well-tolerated in this study and in a previous study (Simons *et al.*, 2000b).

In this model, we have demonstrated for the first time that the bioavailability of sublingual epinephrine increased linearly with a linear increase in the dose, indicating that the absorption follows first-order kinetics. We have also determined for the first time that a 40 mg sublingual epinephrine dose from a

novel, rapidly disintegrating tablet formulation resulted in AUC, C_{max}, and T_{max} values that did not differ significantly from those achieved with a mean dose of 0.34 mg epinephrine intramuscularly. Similar mean (± SEM) AUC, 1420 ± 340 ng/ml/min, and C_{max}, 26.2 ± 6.9 ng/ml, results were reported previously after epinephrine intramuscular injection in the thigh using this rabbit model and similar study conditions (Simons *et al.*, 2000b). Rapid epinephrine absorption is critical for achieving high plasma epinephrine concentrations and fast onset of action by decreasing the release of inflammatory mediators from mast cells and basophils and relieving the symptoms of anaphylaxis (Simons, 2004). In addition, we have confirmed that sublingual epinephrine tablets produced mean T_{max} results similar to those achieved after epinephrine injected intramuscularly, as reported in preliminary studies with an early sublingual epinephrine formulation (Gu *et al.*, 2002).

Plotting the mean plasma epinephrine concentration versus time data of each administered sublingual epinephrine dose and the epinephrine intramuscular dose individually gives a clearer picture of the pattern of epinephrine absorption. After sublingual epinephrine administration, plasma epinephrine concentrations increase immediately (1^{st} small peak) followed by another rapid increase in the form of a 2^{nd} peak, or a series of intermittent peaks. This absorption pattern can be related to the strong vasoconstricting effect of epinephrine in the sublingual mucosa. Initially, epinephrine absorption is almost instantaneous. This is due to rapid transport across the single epithelial cell layer of the mucosa into the interstitial fluid on the basolateral side of the epithelial

cells and then into the venous circulation down the concentration gradient according to Fick's law. The initial rapid absorption of epinephrine induces local vasoconstriction, however, and is therefore followed by a temporary reduction in epinephrine absorption. Subsequent vasodilation is followed by a second and higher plasma epinephrine peak resulting from secondary absorption of the epinephrine which has accumulated in the interstitial fluid. The magnitude of this second peak, and the amount of epinephrine that reaches the systemic circulation, depends on the epinephrine dose and the concentration gradient created across the mucosal epithelial cell layer. Epinephrine absorption after intramuscular injection follows a similar pattern, except that the 1st peak is higher, perhaps indicating that larger amounts of epinephrine are absorbed before the initial vasoconstriction. The subsequent intermittent absorption of epinephrine in the form of small 3rd or 4th peaks is also expected, because some epinephrine is still available at the site of injection.

Epinephrine appears to have a similar intermittent absorption pattern regardless of route of administration, after: sublingual administration in a rabbit model (Gu *et al.*, 2002), intramuscular injection in a rabbit model (Gu *et al.*, 1999; Simons *et al.*, 2000b) and in humans (Simons *et al.*, 2002; Simons *et al.*, 2001b; Simons *et al.*, 1998), subcutaneous injection in a rabbit model (Gu *et al.*, 1999) and in humans (Simons *et al.*, 2001b; Simons *et al.*, 1998), and inhalation in humans (Simons *et al.*, 2000a). It has been suggested previously, based on preliminary experiments with an early sublingual formulation, that the 2nd peak occuring from 90 to 180 min after sublingual administration might be due to

"ongoing absorption from the sublingual mucosa" (Gu *et al.*, 2002) which can be only true for the epinephrine already transported into the mucosal epithelial cells or into the interstitial fluid in the basolateral space. Any epinephrine remaining in the sublingual cavity would be swallowed, and gastrointestinal absorption would be minimal. After oral administration, epinephrine is rapidly metabolized by the catechol-O-methyltransferase in the gastrointestinal tract and by monoamine oxidase in the gastrointestinal tract and in the liver, and is excreted mainly as 3-methoxy-4-hydroxyphenylethylene glycol and 3-methoxy-4-hydroxymandelic acid (Lefkowitz *et al.*, 1996).

The rate of epinephrine absorption as assessed by C_{max} and T_{max}, and the extent of epinephrine absorption as assessed by AUC, from the sublingual tablets was influenced by the magnitude of the epinephrine dose administered. High epinephrine saliva concentrations, the driving force of absorption, were associated with a high epinephrine concentration gradient across the mucosal epithelial cell layer. The increased absorption rate resulted in higher C_{max} at faster T_{max}, and ultimately in higher AUC values in each individual rabbit.

Administration of the 10 mg sublingual tablet may have resulted in epinephrine saliva concentrations that were too low to produce the concentration gradient necessary to achieve absorption across the sublingual mucosa into the venous circulation. The resulting C_{max} and AUC values achieved were not different from, and in some instances even lower than, the endogenous epinephrine concentrations measured after administration of the placebo sublingual tablets. Although the rabbits were conditioned and handled in a

consistent manner, considerable day-to-day variability in mean epinephrine $C_{baseline}$ was observed, as found in previous studies (Gu *et al.*, 1999; Gu *et al.*, 2002; Simons *et al.*, 2000b).

The sublingual administration of 40 mg of epinephrine from a novel rapidly disintegrating tablet resulted in plasma epinephrine concentrations similar to those obtained after epinephrine 0.34 mg intramuscular injection in the thigh. These tablets should be developed further for the potential emergency treatment of anaphylaxis in humans.

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Table 1. Epinephrine bioavailability after sublingual administration of different epinephrine doses and epinephrine intramuscular injection in the thigh

Mean ± SEM*	Sublingual Tablets				EpiPen®
Epinephrine dose (mg)	0	10	20	40	0.3
$AUC_{0\text{-}3\ h}$ (ng/ml/min)	472 ± 126	335 ± 152	801 ± 160	1861 ± 537†	2431 ± 386†
$C_{baseline}$ (ng/ml)	5.5 ± 1.6	0.3 ± 0.1	0.2 ± 0.1	15.4 ± 3.2	9.6 ± 3.5
C_{max} (ng/ml)	6.5 ± 1.3	5.2 ± 2.3	6.6 ± 1.4	31.0 ± 13.1†	50.3 ± 17.1†
T_{max} (min)	-	37 ± 11	31 ± 9	9 ± 2	21 ± 5

*n=5

†$p<0.05$

AUC: area under the plasma concentration versus time curve; $C_{baseline}$: Baseline plasma concentration (endogenous epinephrine); C_{max}: maximum plasma concentration (mean ± SEM of individual C_{max} values from each rabbit, regardless of the time at which C_{max} was achieved); T_{max}: time at which maximum plasma epinephrine concentration was achieved (mean ± SEM of individual T_{max} values from each rabbit).

8.8. Figures

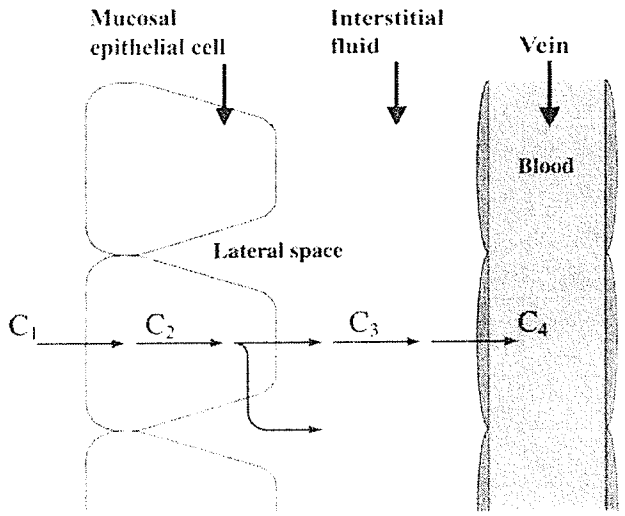

Figure 1: Transcellular absorption of epinephrine from the sublingual cavity into the sublingual veins. C1 epinephrine concentration, which depends on the epinephrine dose and the volume of saliva available; C2 epinephrine concentration within the mucosal epithelial cells; C3 epinephrine concentration in the interstitial fluid; C4 plasma epinephrine concentration. From Bundle: Human Physiology: From Cells to Systems (with CD-ROM and Info-Trac), 5th + Photo Atlas for Biology 5th edition by Sherwood (Sherwood, 2004). © 2004. Adapted and reprinted with permission of Brooks/Cole, a division of Thomson Learning: www.thomsonrights.com.

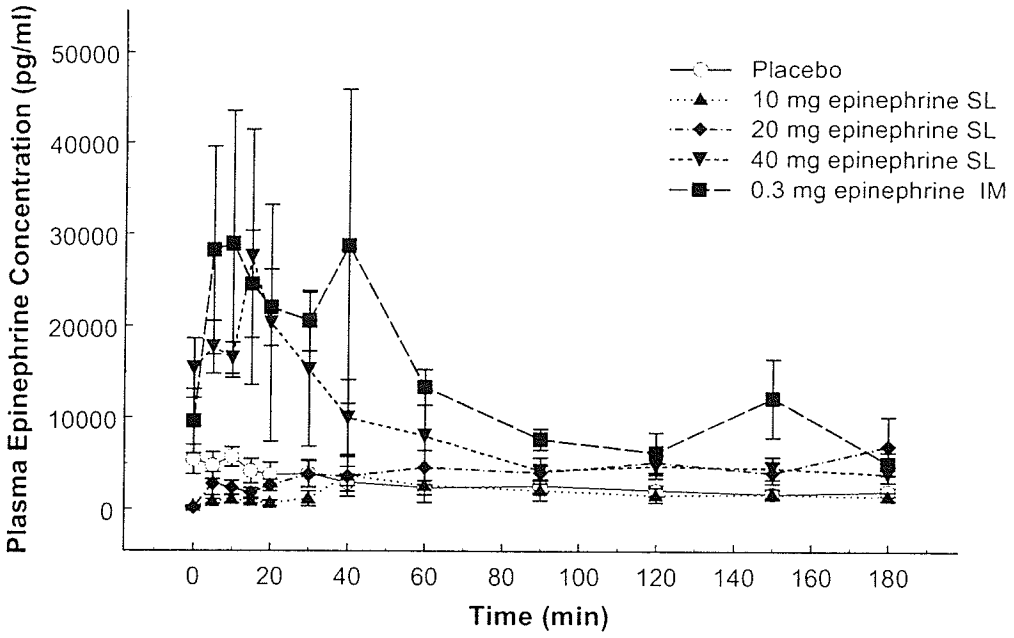

Figure 2: Plasma epinephrine concentration versus time plots after administration of epinephrine or placebo sublingually (SL) and after epinephrine intramuscular injection (IM). Mean (± SEM) AUC, C_{max}, and T_{max} after administration of 40 mg epinephrine sublingual tablets and epinephrine intramuscular injections were not significantly different ($p>0.05$).

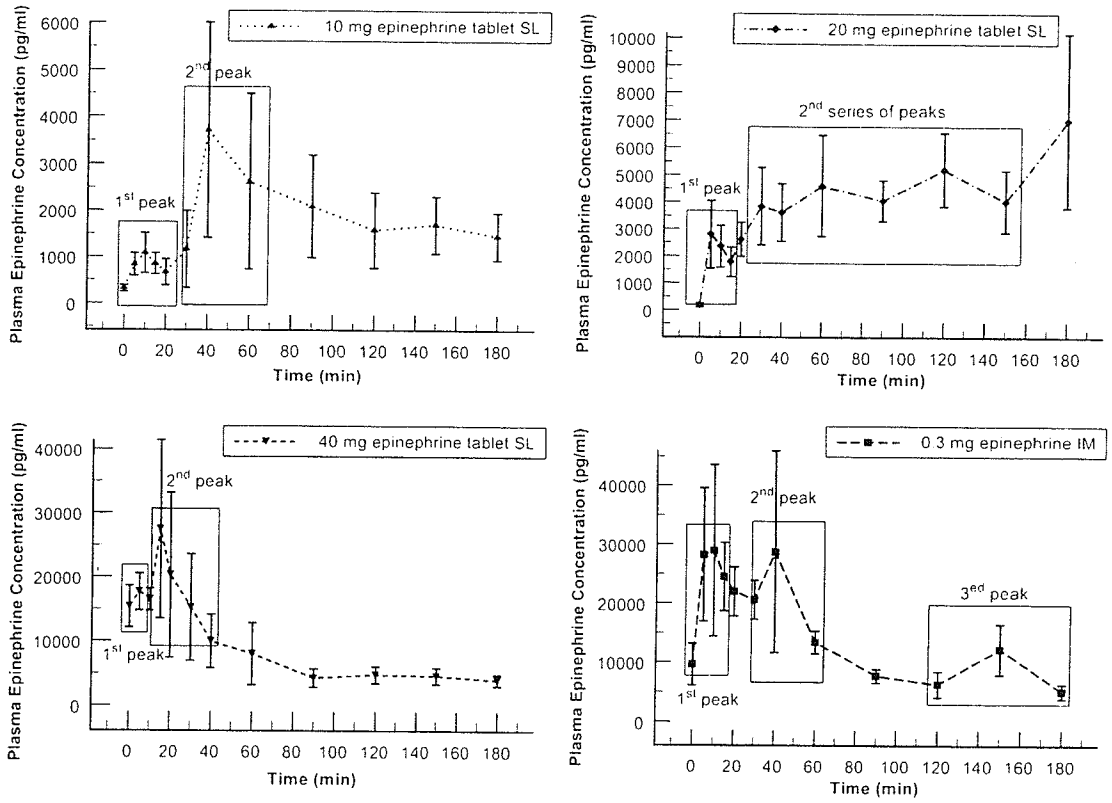

Figure 3: Plasma epinephrine concentration versus time plots after administration of epinephrine or placebo sublingually (SL) and after epinephrine intramuscular injection (IM). Mean (± SEM) AUC, C_{max}, and T_{max} after administration of 40 mg epinephrine sublingual tablets and epinephrine intramuscular injections were not significantly different ($p>0.05$).

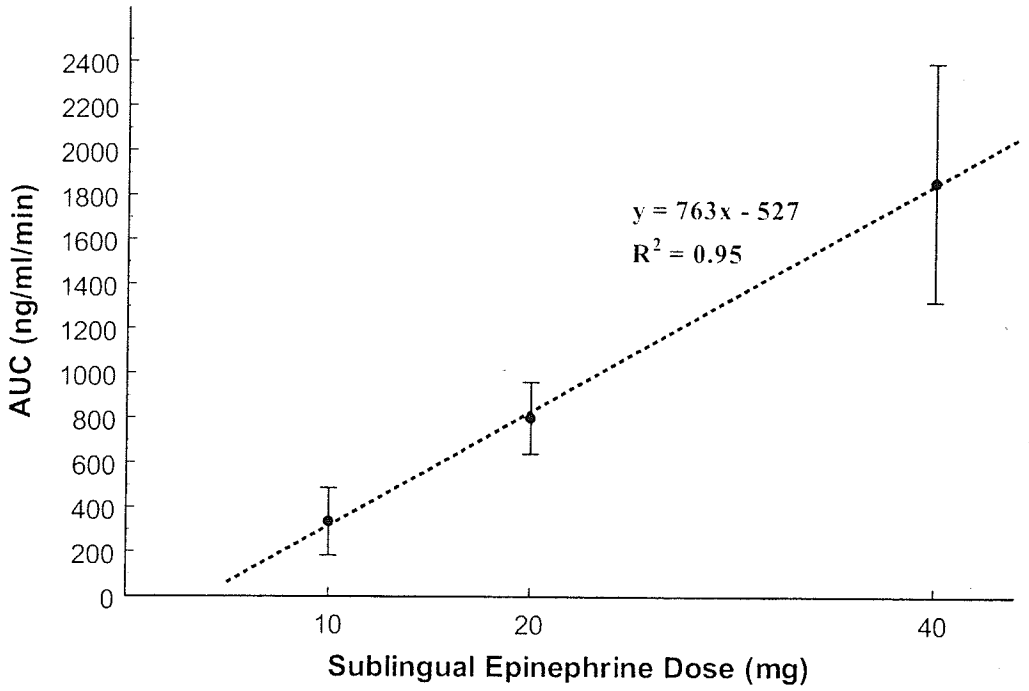

Figure 4: Correlation between epinephrine sublingual dose and the bioavailability of epinephrine. The linear increase in the epinephrine dose administered resulted in a linear increase ($R^2 = 0.95$) in epinephrine bioavailability (AUC). Each data point is expressed as the mean ± SEM of individual AUC values.

CHAPTER IX: Epinephrine for the Treatment of Anaphylaxis: Do All 40 mg Sublingual Epinephrine Tablet Formulations with Similar *In Vitro* Characteristics Have the Same Bioavailability?*

9.1. Abstract

Epinephrine autoinjectors are underutilized in the first-aid emergency treatment of anaphylaxis in the community; so non-invasive sublingual administration of epinephrine is being proposed. In order to determine the effect of changing excipients on the bioavailability of sublingual epinephrine, 4 distinct fast-disintegrating epinephrine 40 mg tablet formulations, A, B, C, and D, were manufactured using direct compression. All formulations were evaluated for tablet hardness (H), disintegration time (DT), and wetting time (WT). In a prospective 5-way crossover study, the 4 sublingual formulations and epinephrine 0.3 mg by intramuscular injection as a control, were tested sequentially in a validated rabbit model. Blood samples were collected before dosing and at intervals afterwards. Epinephrine plasma concentrations were measured using HPLC-EC. All tablet formulations met USP standards for weight variation and content uniformity, and resulted in similar mean H, DT, and WT

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* Reprinted from Biopharm. Drug Dispos., 27, Rawas-Qalaji, M., Simons, E., and Simons, K., Epinephrine for the Treatment of Anaphylaxis: Do All 40 mg Sublingual Epinephrine Tablet Formulations with Similar *In Vitro* Characteristics Have the Same Bioavailability?, 427-35,

(n=6). The area under the curve (AUC), maximum concentration (C_{max}), and time at which C_{max} was achieved (T_{max}) did not differ significantly after the sublingual administration of formulation A and epinephrine 0.3 mg by intramuscular injection. The AUC after B, C, and D were significantly lower ($p<0.05$) than after epinephrine 0.3 mg by intramuscular injection. These results suggest that the selection of excipients used in these tablet formulations can affect the bioavailability of sublingually administered epinephrine.

9.2. Introduction

Epinephrine is the recommended drug of choice for the treatment of anaphylaxis in a dose of 0.3-0.5 mg in adults and 0.01 mg/kg to a maximum of 0.3 mg, in children, given by intramuscular injection in the anterolateral thigh (Lieberman, 2003; McLean-Tooke *et al.*, 2003; Sampson *et al.*, 2006; Simons, 2004). Most anaphylactic reactions occur unexpectedly in the community (Lieberman, 2003; Sampson et al., 2006; Simons, 2004) and for out-of-hospital emergency treatment of anaphylaxis, epinephrine autoinjectors such as EpiPen®, EpiPen Jr® (Dey LP, Nappa, CA), Twinjet 0.3 mg®, and Twinjet 0.15® (Verus Pharmaceuticals, Inc. San Diego, CA) are prescribed. These autoinjectors are underutilized when anaphylaxis occurs (Bock *et al.*, 2001; Gold & Sainsbury, 2000) due to high cost, which limits availability worldwide (Simons, 2005), limitations if multiple doses are required (Korenblat *et al.*, 1999), anxiety associated with the use of needles (Simons, 2004), and errors due to incorrect administration technique (Gold & Sainsbury, 2000; Sicherer *et al.*, 2000). It is

impossible to give accurate doses to infants, or to children weighing < 15 kg or between 20 and 25 kg using the 0.15 and 0.3 mg epinephrine autoinjectors, and alternative methods, such as epinephrine ampules/syringes/needles (Simons *et al.*, 2001) or epinephrine metered-dose inhalers (Simons *et al.*, 2000) are impractical (Simons, 2004).

The readily accessible, convenient sublingual route of administration has long been used to administer medications such as nitroglycerine and was also used in the 1970's for the administration of isoproterenol. Sublingual administration is a promising non-invasive alternative route for epinephrine administration (Rawas-Qalaji *et al.*, 2006a). Drugs administered sublingually bypass potential metabolic conversion in the gastrointestinal tract and hepatic first-pass metabolism, and reach the systemic circulation in a pharmacologically active form (Cunningham *et al.*, 1994; Kroboth *et al.*, 1995; Price *et al.*, 1997). Lipophilic drugs with a low molecular weight such as epinephrine are likely absorbed across the sublingual mucosa into the venous circulation by transcellular diffusion (Birudaraj *et al.*, 2005), a mechanism driven by the concentration gradient (Rawas-Qalaji *et al.*, 2006a).

Tablet formulations that disintegrate or dissolve rapidly in the sublingual cavity are required in order to enhance the availability of epinephrine for rapid absorption into the blood vessels in the sublingual mucosa. Using a novel fast-disintegrating tablet formulation, the sublingual epinephrine dose required to achieve plasma epinephrine concentrations similar to those obtained after the intramuscular injection of 0.3 mg epinephrine from an EpiPen® was determined

to be 40 mg (Rawas-Qalaji *et al.*, 2006a). The effect of changing tablet excipients (non-medicinal ingredients) on the bioavailability of epinephrine sublingual tablets has not yet been described.

The aim of this study was to evaluate the effect of changing excipients on the epinephrine sublingual bioavailability from four different tablet formulations which have similar *in vitro* tablet characteristics, in comparison to epinephrine 0.3 mg by intramuscular injection in the thigh. The study was performed using a validated rabbit model that has been used previously to compare the rate and extent of epinephrine absorption after epinephrine administration by several different routes, such as intravenous, intramuscular, subcutaneous, pulmonary, and sublingual routes (Gu *et al.*, 1999; Rawas-Qalaji *et al.*, 2006a).

9.3. Materials and Methods

9.3.1. Materials

(-)-Epinephrine (+) bitartrate, (-)-3,4-dihydroxy-α-[(methylamino)methyl] benzyl alcohol (+)-tartrate (1:1) salt, was purchased from Sigma-Aldrich (St. Louis, MO). The following excipients were kindly supplied by the manufacturers and used as received: Ceolus® (microcrystalline cellulose), type PH-301, PH-M-06, and KG-802 (Asahi Kasei Chemicals Corp, Tokyo, Japan), RxCipient® (calcium silicate), type FM1000 (Huber Engineered Materials, Havre de Grace, Maryland), and Pearlitol® (mannitol), type 400 DC (Roquette America, Inc., Keokuk, IA), as fillers; low-substituted hydroxypropyl cellulose, type LH11 (Shin-Etsu Chemical Co, Tokyo, Japan) and Polyplasdone®

(crospovidone), type XL-10 (ISP Technologies, INC., Wayne, New Jersey), as superdisintegrants; Pharmaburst® (patent formula), is a ready to use formula for fast-disintegrating tablets (SPI Pharma, New Castle, DE); RxCipient® (silicon dioxide), type GL200 (Huber Engineered Materials, Havre de Grace, Maryland), as a glidant; PRUV® (sodium stearyl fumerate) by JRS Pharma LP (Patterson, NY), and magnesium stearate was purchased from Mallinckrodt Baker (Phillipsburg, NJ); as lubricants.

9.3.2. Preparation of Tablets

Four tablet formulations, A, B, C, and D containing 48.5% of epinephrine bitartrate, equivalent to 40 mg of epinephrine, were prepared by direct compression (Table 1). The total weight of the compressed tablets was maintained at 150 mg. These tablets were prepared by mixing the pre-weighted excipients and epinephrine using a three dimensional manual mixer (Inversina®, Bioengineering AG, Switzerland). The microcrystalline cellulose : low-substituted hydroxypropyl cellulose ratio in formulations A and B was 9:1 (Bi *et al.*, 1996; Ishikawa *et al.*, 2001). All of the magnesium stearate and sodium stearyl fumerate were added just before the end of mixing.

Each tablet formulation was compressed using an 11/32 inch die, a flat, scored face, bevel edge upper punch, and a flat, bevel edge lower punch. The tablets were compressed at a pre-selected compression force based on results from our previous study (Rawas-Qalaji *et al.*, 2006b) using a Manesty® – F3 single-punch tablet press machine (Liverpool, UK). All tablets were formulated

in the manufacturing laboratory of the Faculty of Pharmacy at the University of Manitoba.

9.3.3. In Vitro Evaluation of Tablet Characteristics

Each batch of 200 tablets was collected into a stainless steel beaker. Tablet weight variation, drug content uniformity, and friability was measured using USP methods and criteria (USP/NF, 2003a, 2003b). Drug content was analyzed using a high performance liquid chromatography (HPLC) system with ultra violet (UV) detection (Waters Corp., Milford, MA) and tablet friability was measured using USP Friability instrument (Pharma Test Apparatebau GmbH, Hainburg, Germany). Six tablets were selected randomly from each formulation batch and tested for tablet hardness, disintegration time, and wetting time. The mean ± standard error (SEM) and percentage of coefficient of variation (CV %) were calculated.

9.3.3.1. Hardness (H)

The H or the crushing tolerance of tablets was measured by an Erweka® hardness tester (Heusenstamm, Germany).

9.3.3.2. Disintegration Time (DT)

A novel, relatively simple method with rigorous requirements was developed to evaluate the DT of rapidly disintegrating tablets, which has been described previously (Rawas-Qalaji *et al.*, 2006b).

9.3.3.3. Wetting Time (WT)

Tablet WT was measured by a procedure similar to that reported by Bi *et al* (Bi *et al.*, 1996) with slight modifications as described previously (Rawas-Qalaji *et al.*, 2006b).

9.3.3.4. Effect of Water-Soluble Excipients on Epinephrine Solubility

The dissolution of 7.3 mg of epinephrine bitartrate was evaluated in 100 µL of water and 100 µL of a saturated solution of mannitol, equivalent to 40 mg of epinephrine dissolving in 1 mL of saliva (1 mL saliva volume was based on the normal salivary secretion in humans, 0.2 mL/min (Diem *et al.*, 1971), over 5 min). Dissolution was monitored over 5 min using a microscope (10^{x4} power) (Nikon YS100, Nikon Canada Inc., ON, Canada) equipped with a digital camera (Sony 3-CCD, DXC-390P, Sony Electronics Inc., NJ) using Northern Eclipse V6.0 software (Empix Imaging, Inc, ON, Canada).

9.3.4. Animal Studies

All animal studies were conducted according to current guidelines published by the Canadian Council on Animal Care (Olfert *et al.*, 1993) and were approved by the University of Manitoba Protocol Management and Review Committee.

Using a prospective, controlled, 5-way crossover sequential study design, five New Zealand white rabbits (mean weight ± SEM, 4.7 ± 0.1 kg) were

investigated on five different study days at least four weeks apart, using a protocol described previously (Gu *et al.*, 1999). Each rabbit received epinephrine 40 mg sublingual tablet using each formulation, and epinephrine 0.3 mg intramuscular in the right thigh using an EpiPen® of the same lot number. The procedure of sublingual epinephrine tablet administration to rabbits was described previously (Rawas-Qalaji *et al.*, 2006a).

After epinephrine intramuscular, the solution remaining in the EpiPen® autoinjector was evacuated into a polystyrene test tube, sealed and frozen at –20 °C, to be analysed for epinephrine content using HPLC system with UV detection (Waters Corp., Milford, MA).

9.3.5. Measurement of Plasma Epinephrine Concentrations

An indwelling catheter (OPTIVA® 22G 1", Johnson & Johnson Medical, Arlington, TX) was inserted into an ear artery 30 min before dosing. A 2 mL blood sample was obtained immediately before dosing and at 5, 10, 15, 20, 30, 40, 60, 90, 120, 150, and 180 minutes afterward.

Blood samples were refrigerated within 1 hour of sampling and centrifuged at 4 °C. Plasma was frozen at –20 °C. Before analysis, the plasma was thawed at room temperature and epinephrine was extracted by a solid-liquid extraction process, with an efficiency of 84%. Epinephrine concentrations were measured using an HPLC system with electrochemical detection (Waters Corp., Milford, MA) (Ganhao *et al.*, 1991; Hjemdahl, 1984, 1987). Two calibration curves with two different epinephrine concentration ranges were prepared. The low-

range calibration curve was linear over the range of 0.1 to 1.0 ng/mL, with a coefficient of variation of 0.8% at 0.1 ng/mL and 1.5% at 1.0 ng/mL. The high-range calibration curve was linear over the range of 1.0 to 10.0 ng/mL, with a coefficient of variation of 5.0% at 1.0 ng/mL and 1.2% at 10.0 ng/mL.

9.3.6. Data Analysis

Mean (± SEM) maximum plasma epinephrine concentrations (C_{max}), the times at which C_{max} was achieved (T_{max}), and the area under the plasma concentration versus time curves (AUC) were calculated by a trapezoidal rule from the plasma epinephrine concentration versus time plots of each individual rabbit using WinNonlin® 5.0 (Pharsight, Mountain View, CA). The AUC, C_{max}, and T_{max} values for each rabbit were compared by using repeated-measures ANOVA, Tukey-Kramer tests, and paired Students' t-test using NCSS Statistical Analysis Software (NCSS, Kaysville, UT). Differences were considered to be significant at $p < 0.05$.

9.4. Results

9.4.1. *In Vitro* Results

The powders from all four formulations had good mixing, flowability, and compressibility characteristics. Tablets manufactured from each formulation were within USP specifications for weight variation and drug content uniformity (USP/NF, 2003b).

The mean (± SEM) hardness, disintegration time, and wetting time results of the four tablet formulations are summarized in Table 2. Tablet hardness was similar for all four formulations and ranged from 1.5 ± 0.1 kg to 2.6 ± 0.1 kg. The disintegration and wetting times were less than 15 sec and 60 sec, respectively, for all four tablet formulations. Tablets from formulations A and C met the USP standards for tablet friability (USP/NF, 2003a) (Table 2).

The dissolution of 7.3 mg of epinephrine bitartrate in 100 µL of water, the control, was complete in less than 3 min (Figure 1) when compared with dissolution of 7.3 mg of epinephrine bitartrate in 100 µL of a saturated solution of mannitol, which was incomplete after 5 min (Figure 2).

9.4.2. *In Vivo* Results

The mean (±SEM) epinephrine dose injected using EpiPen® autoinjectors was 0.34 ± 0.002 mg, calculated by multiplying the epinephrine concentration, measured in the evacuated EpiPen® solutions, by the stated injected volume (0.3 mL). Mean epinephrine tablet doses were 38.15 ± 0.51, 35.79 ± 0.30, 39.20 ± 0.29, and 39.34 ± 0.28 mg for formulations A, B, C, and D respectively, measured using the USP content uniformity standard test (Table 3).

Mean (±SEM) plasma epinephrine concentration versus time plots after the administration of epinephrine 40 mg sublingual tablets of each formulation and epinephrine 0.3 mg by intramuscular injection are shown in Figure 3. Mean (±SEM) AUC, $C_{baseline}$ (endogenous), C_{max}, and T_{max} values after the administration of epinephrine 40 mg sublingual tablets of each formulation

and epinephrine 0.3 mg by intramuscular injection are shown in Table 3. No adverse effects were observed.

Mean (±SEM) AUC after the administration of epinephrine 40 mg sublingual tablets of formulation A (1861 ± 537 ng/mL/min) and epinephrine 0.3 mg by intramuscular injection (2431 ± 386 ng/mL/min) did not differ significantly. Mean AUC after the administration of epinephrine 40 mg of formulation B (615 ± 87 ng/mL/min), formulation C (606 ± 149 ng/mL/min), and formulation D (646 ± 202 ng/mL/min) sublingual tablets were significantly lower than after epinephrine 0.3 mg by intramuscular injection (2431 ± 386 ng/mL/min).

Mean (±SEM) C_{max} values after epinephrine 40 mg sublingual tablets of formulation A (31.0 ± 13.1 ng/mL) and epinephrine 0.3 mg by intramuscular injection (50.3 ± 17.1 ng/mL) did not differ significantly. Mean C_{max} values after epinephrine 40 mg of formulation B (6.0 ± 0.9 ng/mL), formulation C (7.1 ± 1.6 ng/mL), and formulation D (6.7 ± 3.2 ng/mL) sublingual tablets were significantly lower than after epinephrine 0.3 mg by intramuscular injection (50.3 ± 17.1 ng/mL).

Mean (±SEM) T_{max} after the administration of epinephrine 40 mg of formulation A (9 ± 2 min), formulation B (28 ± 10 min), formulation C (27 ± 9 min), and formulation D (16 ± 4 min) sublingual tablets, and epinephrine 0.3 mg by intramuscular injection (21 ± 5 min) did not differ significantly.

9.5. Discussion

Interest in the sublingual route as a readily accessible and non-invasive route of administration has been increasing recently for a wide range of medications (Bredenberg *et al.*, 2003; Cunningham *et al.*, 1994; Glover *et al.*, 2002; Guez, 2003; Kroboth *et al.*, 1995; Price *et al.*, 1997; Saxena *et al.*, 2005). The high vascularity of the sublingual mucosa facilitates rapid drug absorption directly into the venous circulation through the sublingual and frenular veins, bypassing the gastrointestinal tract, the hepatic portal circulation, and hepatic first-pass metabolism. In comparison to the extremely limited range of doses currently available in epinephrine autoinjectors, sublingual tablets can be formulated in a wide range of epinephrine doses to provide accurate doses for individuals over a wide range of ages and body weights (Rawas-Qalaji *et al.*, 2006a). Tablets are easy to carry and unobtrusive to self-administer sublingually. Multiple doses could be readily available. Tablets should be less expensive to produce than the autoinjectors units, and, unlike autoinjectors, are easy to dispose of in a safe and ecologically acceptable manner.

It has been demonstrated for the first time that the bioavailability of epinephrine following the sublingual administration of a 40 mg dose from different fast-disintegrating tablet formulations might be affected considerably by the composition of the excipients in the tablets. All four tablet formulations met the USP standards for content and weight variation and resulted in similar disintegration and wetting times. However, only formulation A resulted in AUC, C_{max}, and T_{max} values that did not differ significantly from those obtained after a

mean dose of 0.34 mg epinephrine by intramuscular injection. Although the T_{max} values obtained after the sublingual administration of formulation A did not differ significantly from those after the administration of 0.34 mg epinephrine by intramuscular injection, there was evidence of a shorter and more desirable T_{max} after the administration of formulation A when compared to the intramuscular injections. Tablets from formulations A and C passed the USP friability test. Although tablets from formulations B and D did not pass the USP friability test, they had sufficient hardness for handling during the sublingual administration in the animal model.

In this study, the differences in the epinephrine bioavailability from the four tablet formulations are a result of the type of excipients used in these formulations. The rate-limiting step for epinephrine absorption following sublingual administration is the rate of dissolution. The epinephrine bitartrate crystals used in these tablet formulations are very water-soluble (1 gm in 3 mL of water (Keefe, 2000)). Dissolution occurred rapidly and was complete in less than 3 min (Figure 1). The rate of dissolution of epinephrine bitartrate can be influenced by the presence of other water-soluble excipients. The mannitol, a very water-soluble excipient (1 gm in 5.5 mL of water (Hanson, 2000)), was used in formulation B at 24.74% and in formulation C at 26.0% of tablet weight. Epinephrine bitartrate dissolution in a saturated solution of mannitol was slow and incomplete at the end of 5 min (Figure 2), the length of time for which the tablet was held under the rabbit's tongue. The mannitol in formulations B and C might reduce the epinephrine bitartrate rate and extent of dissolution, especially

in the limited saliva volumes available in the sublingual cavity, and therefore might reduce epinephrine bioavailability from these formulations. Formulation D was prepared using a patented excipient of unknown composition.

The AUC, and C_{max} values after the 40 mg sublingual epinephrine dose in formulations B, C, and D were significantly lower than those following a mean dose of 0.34 mg epinephrine injected intramuscularly. They were of the same order of the values after a 20 mg sublingual epinephrine dose of formulation A (801 ± 160 ng/mL/min and 6.6 ± 1.4 ng/mL, respectively) which was used in our previous study (Rawas-Qalaji *et al.*, 2006a). Lower AUC and C_{max} values indicate that epinephrine bitartrate dissolution might have been decreased by the presence of mannitol in formulations B and C.

It is unlikely that epinephrine diffusion across the sublingual epithelial mucosa into the venous circulation is influenced by any of the excipients used in these four tablet formulations. Monosaccharides are absorbed by a secondary active transport utilizing sodium cotransporters (Sherwood, 2004) and should not interfere with epinephrine transcellular passive absorption (Birudaraj *et al.*, 2005). Water-insoluble excipients will not be absorbed because they do not dissolve in saliva.

The formulations containing very water-soluble excipients, such as mannitol and other sugars, could possibly delay the dissolution of epinephrine bitartrate and reduce the sublingual bioavailability of epinephrine. The sublingual administration of 40 mg of epinephrine from the novel water-insoluble, rapidly disintegrating tablet, formulation A resulted in plasma epinephrine concentrations

similar to those obtained after epinephrine 0.34 mg by intramuscular injection in the thigh. These tablets should be developed further for the potential first-aid emergency treatment of anaphylaxis in humans.

9.6. References

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9.7. Tables

Table 1. Composition of the four tablet formulations of epinephrine*

Ingredient Weight %	Tablet Formulations			
	A	B	C	D
Epinephrine Bitartrate	48.51	48.51	48.51	48.51
Microcrystalline Cellulose (PH-301)	44.54	-	-	-
Microcrystalline Cellulose (PH-M-06)	-	22.27	-	-
Microcrystalline Cellulose (KG-802)	-	-	12.87	-
Calcium Silicate	-	-	10.55	
Pharmaburst ®	-	-	-	49.49
Low-Substituted Hydroxypropyl Cellulose (LH1	4.95	2.47	-	-
Crospovidone		-	1.3	-
Mannitol	-	24.74	26.00	-
Silicon Dioxide	-	-	0.26	-
Magnesium Stearate	2.00	2.00	0.51	-
Sodium Stearyl Fumerate	-	-	-	2.00

*Tablet weight was 150 mg.

Table 2. The hardness, disintegration time, wetting time, and friability of the four tablet formulations*†

Formulations	*In Vitro* Tablets Characteristics						
	H	CV	DT	CV	WT	CV	F
A	2.4 ± 0.1	12.4	13.5 ± 0.2	4.1	26.2 ± 1.8	17.0	0.6
B	1.5 ± 0.1	16.9	13.2 ± 0.8	14.7	47.3 ± 3.3	16.9	13.4
C	2.4 ± 0.1	7.5	9.3 ± 0.5	13.0	14.3 ± 0.6	9.5	0.3
D	2.6 ± 0.1	4.3	8.3 ± 0.3	9.8	26.5 ± 2.0	18.2	6.5

*mean ± SEM (n=6).

†H indicates tablet hardness (kg); CV, coefficient of variation (%); DT, disintegration time (sec); WT, wetting time (sec); F: friability (%) (USP limits ≤ 1%).

Table 3. Epinephrine bioavailability after 40 mg sublingual epinephrine administration from four different tablet formulations and epinephrine 0.3 mg intramuscular (IM) injection in the thigh

	Sublingual Tablets				IM Injection
Mean ± SEM*	A	B	C	D	EpiPen®
Epinephrine dose (mg)	38.15 ± 0.51	35.79 ± 0.30	39.20 ± 0.29	39.34 ± 0.28	0.34 ± 0.002
$AUC_{0\text{-}3\ h}$ (ng/mL/min)	1861 ± 537	615 ± 87†	606 ± 149†	646 ± 202†	2431 ± 386
$C_{baseline}$ (ng/mL)	15.4 ± 3.2	4.2 ± 0.7	11.2 ± 7.5	3.5 ± 1.4	9.6 ± 3.5
C_{max} (ng/mL)	31.0 ± 13.1	6.0 ± 0.9†	7.1 ± 1.6†	6.7 ± 3.2†	50.3 ± 17.1
T_{max} (min)	9 ± 2	28 ± 10	27 ± 9	16 ± 4	21 ± 5

*n=5.

†$p < 0.05$ compared to IM injection.

AUC: area under the plasma concentration versus time curve; $C_{baseline}$: Baseline plasma concentration (endogenous epinephrine); C_{max}: maximum plasma concentration (mean ± SEM of individual C_{max} values from each rabbit, regardless of the time at which C_{max} was achieved); T_{max}: time at which maximum plasma epinephrine concentration was achieved (mean ± SEM of individual T_{max} values from each rabbit).

9.8. Figures

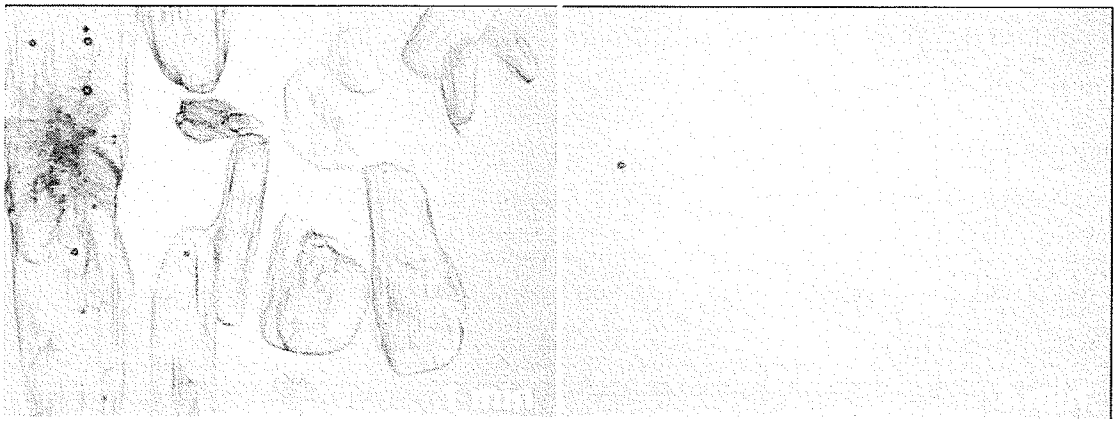


Figure 1: Photomicrograph of the dissolution of epinephrine bitartrate crystals in water over 3 min.

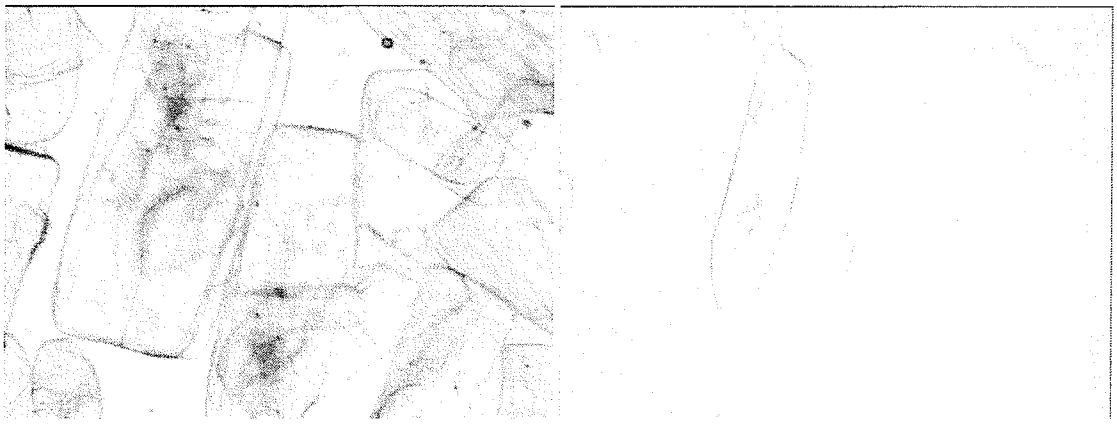


Figure 2: Photomicrograph of the dissolution of epinephrine bitartrate crystals in a saturated solution of mannitol over 5 min.

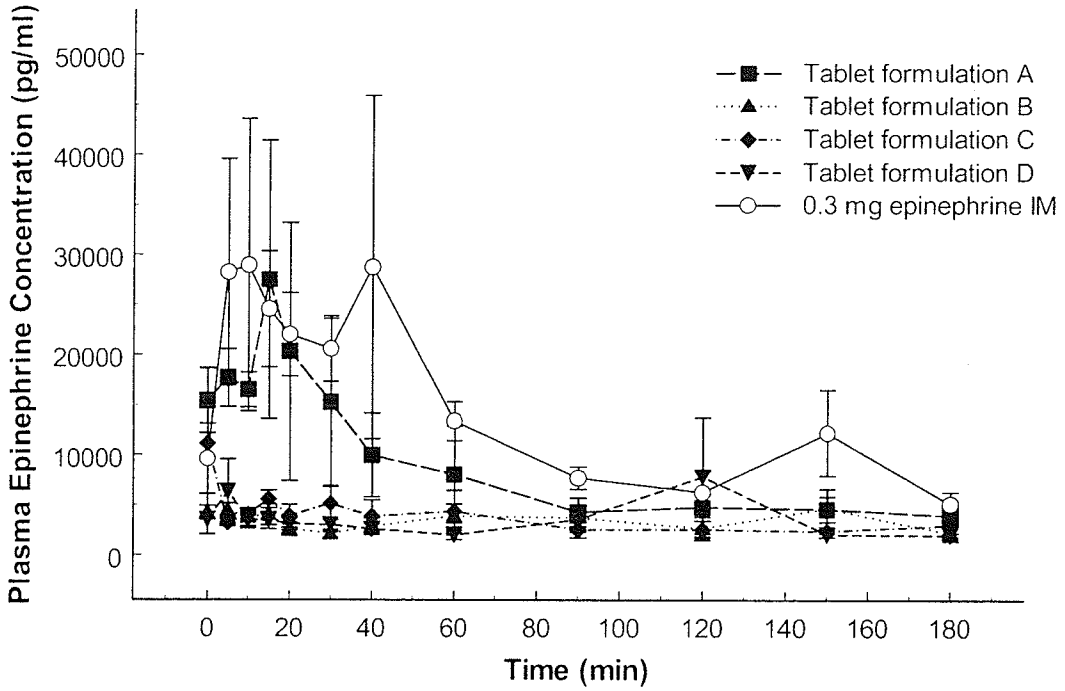


Figure 3: Plasma epinephrine concentration versus time plots after administration of epinephrine sublingually of four different tablet formulations, and after epinephrine intramuscular injection (IM).

CHAPTER X: Conclusions

Novel fast-disintegrating sublingual epinephrine tablets were developed for the potential out-of-hospital emergency treatment of anaphylaxis. These tablets were evaluated in a systematic series of experiments in order to characterize all of the parameters that might affect the rate and extent of epinephrine absorption by this route of administration.

Epinephrine is stable in aqueous solutions at non-optimal pH and in human saliva for at least 20 minutes. The sublingual route is therefore feasible for the sublingual administration of epinephrine.

Changing the shape and dimensions of these tablets in order to enhance their suitability for sublingual administration can be achieved without adversely affecting the disintegration and wetting times, when tablet hardness was maintained below the critical point that results in plastic deformation of the powder particles.

Tablets with drug loads from 0% to 48% epinephrine bitartrate, equivalent to 0 to 40 mg of (-)-epinephrine, can be formulated with optimal disintegration and wetting times suitable for sublingual administration, without compromising the tablet hardness and friability.

These fast-disintegrating sublingual tablets containing a range of epinephrine doses, 10, 20 and 40 mg, are stable yet release epinephrine rapidly

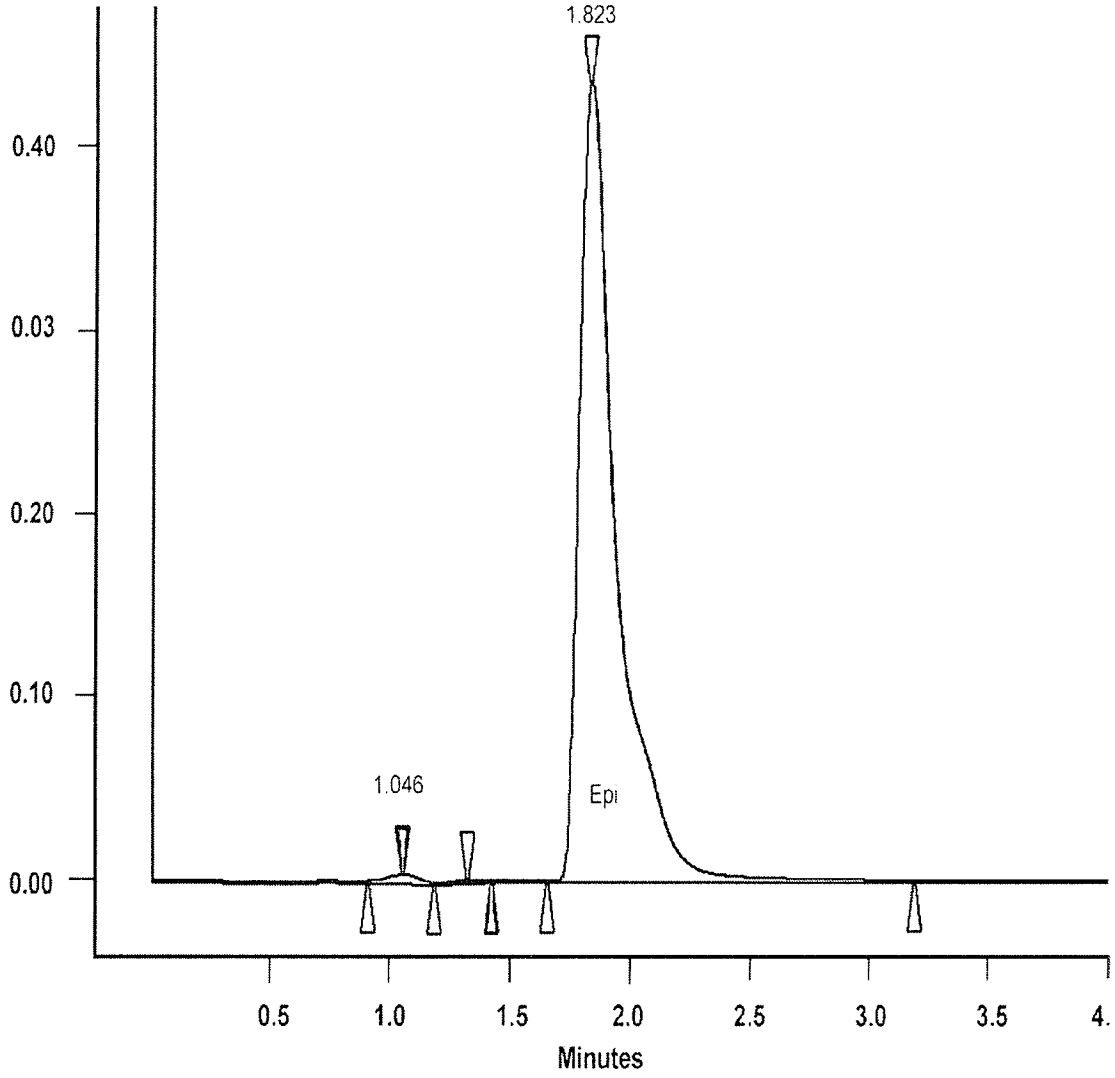
in saliva. They are therefore potentially useful for the emergency treatment of anaphylaxis.

In a validated rabbit model, the extent of sublingual epinephrine absorption increased linearly with increases in the doses administered, indicating that the absorption follows first-order kinetics.

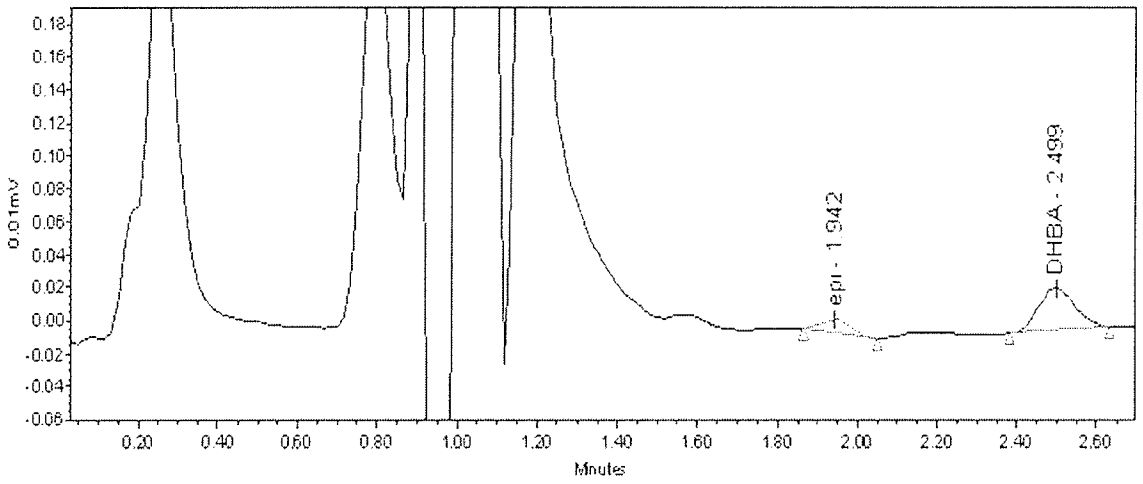
The sublingual administration of epinephrine 40 mg from these novel water-insoluble, rapidly disintegrating tablets, resulted in a rate and an extent of epinephrine absorption similar to that measured after the intramuscular injection of epinephrine 0.3 mg in the thigh.

The 40 mg sublingual epinephrine dose from this novel tablet formulation is bioequivalent to epinephrine 0.3 mg intramuscular injection and now needs to be studied in humans.

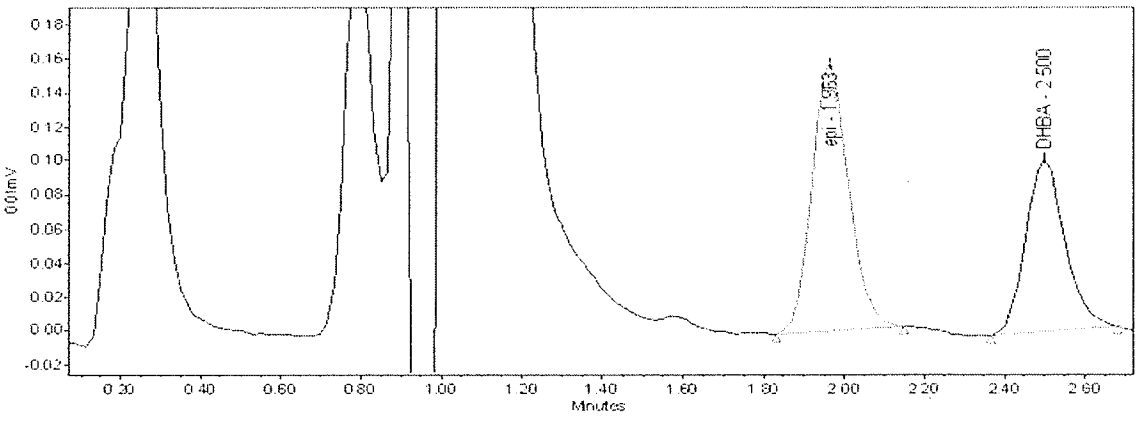
Appendix



Chromatogram 1: A representative HPLC chromatogram of epinephrine from a calibration curve used for the analysis of epinephrine from aqueous solutions.



Chromatogram 2: A representative HPLC chromatogram of epinephrine and DHBA from a low range calibration curve used for the analysis of epinephrine from plasma.



Chromatogram 3: A representative HPLC chromatogram of epinephrine and DHBA from a high range calibration curve used for the analysis of epinephrine from plasma.