THERMOREVERSIBLE IN SITU GELLING FORMULATION OF CARBAMAZEPINE FOR INTRANASAL DELIVERY: PREPARATION, CHARACTERIZATION AND EX-VIVO STUDY

BY

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ABSTRACT

Carbamazepine (CBZ) is used to treat epilepsy and is an anti-convulsant requiring the drug to reach the brain to exert their pharmacological response. The presence of blood-brain barrier (BBB) poses a great challenge for any drugs to reach the receptors in the brain especially when the drugs are administered by oral route. Over the past decade, intranasal (IN) delivery has been increasingly considered as an alternative route of drug delivery. This is because the nasal mucosa has a large surface area and high blood flow that allows better bioavailability. However, this route poses high variability in drug absorption due to short retention time of dosage form on nasal mucosa complicated by naso-ciliary clearance mechanism. This research aims to investigate ability of a novel dosage form i.e. a thermoreversible in situ gelling system on absorption of CBZ via IN route. Briefly, a CBZ thermoreversible gel was developed by mixing 15-20% Poloxamer 407 (P407) with 0.15-0.25% τ-Carrageenan (ι-Cg) by cold method to 3% CBZ solution (weight ratio). The formulations takes liquid state when stored at 2-8˚C and gel at nasal temperatures (32-34°C). The gelation temperature and pH for the in situ gels were 26-36 °C and 4.5-6.5 respectively. CBZ compatibility in formulation was determined by a developed ATR-FTIR method. Rheology study of the gel by a controlled (CR) ramp test showed pseudoplasticity and distinct shear-thinning with increasing shear stress. CT3 Texture analyzer was used to determine the mucoadhesion strength of the gel using goat nasal mucosa. Monopolymeric formulations containing 18% and 20% showed greater mucoadhesive strength (<300,000 dyne/cm²) as opposed to binary mixtures containing P407 and ι-Cg (T10-T18). In vitro was conducted with a dialysis membrane and formulations containing only P407 had greater release compared to those with both P407 and ι-Cg in 24h. Ex-vivo drug permeation study through goat nasal mucosa was done using Franz diffusion cell. Permeation flux was shown to be greater in case of thermoreversible gel without ι-Cg. Drug permeation studies have indicated that the permeation decreases with increasing ι-Cg concentration. Formulation containing 18% P407 + 0.20% ι-Cg showed the highest cumulative drug permeation at 243.94 ug/cm². The gel formula demonstrated high flux and correlated well with the mucoadhesive strength. The use of ι-Cg is to ensure prolonged contact of the gel and nasal mucosa. The increasing concentrations of binary mixtures showed sustained-release behavior in in-vitro study. From ex-vitro studies, it can be concluded that the in situ gelling system can result in high nasal epithelial permeation with a sustained-release behavior, which have high potential to successfully deliver drugs systemically for a prolonged period of time. The brain distribution study of the drug using the developed in-situ gel is subjected to future in-vivo study in animal model.

خلاصة البحث

الأدوية مثل كاربامازيبين (CBZ) التي تستخدم لعلاج اعتلال الأعصاب السكري, والصرع, وآلام الاعتلال العصبي بعد العمل الجراحي تتطلب الوصول إلى الدماغ لممارسة استجابتها الدوائية. إن وجود الحاجز الدموي الدماغي (BBB) يشكل تحدياً كبيراً لأي دواء للوصول إلى المستقبلات في الدماغ وخاصة عندما يتم إعطاء الأدوية عن طريق الفم. على مدى العقد الماضي, اعتبر ايصال الدواء عبر الأنف (IN) كبديل لايصاله عن طريق الوريد. وذلك بسبب المساحة السطحية الكبيرة للغشاء المخاطي للأنف والتي تسمح ببدء سريع للفِعْل العلاجي, وإمكانية ايصال الدواء مباشرةً إلى الدماغ أو الجهاز العصبي المركزي (CNS) عبر المسار الشمي, وتجاوز تأثير المرور الكبدي الأول. ومع ذلك, فإن هذا الطريق لديه تباين كبير في امتصاص الدواء و ذلك بسبب قصر زمن استبقاء الشكل الجرعي على الغشاء المخاطي للأنف بشكل مختلط مع آلية الإزالة الهدبية الأنفية. يهدف هذا البحث إلى التحقق من قدرة شكل جرعي جديد, نظام متهلم مُتشكل في الموضع عكوس حرارياً, على امتصاص الأدوية عن طريق الأنف. كان الدواء النموذجي المستخدم هو الكاربامازبين. باختصار, تم تطوير هلام عكوس حرارياً للكاربامازبين عن طريق خلط 15-20 % من بولوكسامير 407 مع 0.15-0.25 % من كاراجينان (τ-Cg) بالطريقة الباردة إلى محلول كاربامازبين (3%) (نسبة وزنية). أصبحت الصيغ سائلة عند تخزينها في 2-8 درجة مئوية. وكانت درجة حرارة الهلام ودرجة الحموضة و القلوية, للهلامات المُتشكلة في الموضع 35-37 درجة مئوية و 4.5-6.5 على التوالي. أظهرت دراسة الجريان للهلام عن طريق اختبار ramp المراقب أن الهلام زائف اللدونة ذو قوام مترقق بالقص عند زيادة إجهاد القص. تم استخدام محلل الملمس CT3 لتحديد قوة التصاق الهلام بالغشاء المخاطي وذلك باستخدام الغشاء المخاطي الأنفي للماعز. أُجريت دراسة نفاذ للدواء السابق خارج الجسم الحي عن طريق الغشاء المخاطي الأنفي للماعز باستخدام خلايا انتشار فرانز. قُيمت كمية الكاربامازبين في الصيغة و كمية الكاربامازبين النافذة خارج الجسم الحي من العينات بواسطة تطوير طريقة نظام فورييه للتحليل الطيفي بتحويل الأشعة تحت الحمراء. تبين أن تدفق النفاذ يكون أكبر في حالة الهلام العكوس حرارياً الغير الحاوي على كاراجينين مقارنة مع ذلك الحاوي على الكارجينين. وقد أوضحت دراسات النفاذ للدواء أن النفاذية تتناقص مع زيادة تركيز الكاراجيننين. أَظهرت الصيغة ι-Cg 0.20% + P407 18% أكبر نفاذ دوائي تراكمي عند 181.15 ميكروغرام / سم². أظهرت صيغة الهلام تدفقاً عالياً مرتبطاً جيداً مع قوة الالتصاق بالغشاء المخاطي. من الدراسات التي أجريت في الزجاج, يمكن أن نستنتج أن نظام التهلم المُتشكل في الموضع يمكن أن يؤدي إلى نفاذ عالٍ خلال الظِهارة الأنفية مع نمط مضبوط التحرر, وهو ما ينطوي على إمكانية عالية لإيصال الدواء بنجاح إلى الدماغ بكفاءة ولفترة أطول من الزمن. أَظهرت الصيغة أيضًا تأثير غير سام على الغشاء المخاطي الأنفي للماعز في تجربة خارج الجسم الحي. تخضع دراسة التوزيع الدماغي للدواء باستخدام الجل المتشكل في الموضع المُطور لدراسة مستقبلية في نموذج حيواني.

APPROVAL PAGE

I certify that I have supervised and read this study and that in my opinion, it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a thesis for the degree of Master of Pharmaceutical Science (Pharmaceutical Technology).

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

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Signature Date

This thesis is dedicated to the bravest person I know: my grandmother.

May I always have her brave spirit.

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CHAPTER ONE

INTRODUCTION

1.1 INTRANASAL DELIVERY

Intranasal (IN) is lying within or administering via nasal structures. The early 1980s saw the introduction of nasal route as a promising alternative for systemic therapy (Appasaheb, 2013). Over the past few decades, the nasal route has been gaining attention as a promising route of drug administration for systemic therapy. Up until this point, the oral and parenteral routes have been the most popular routes for drug delivery before alternative routes of drug delivery starting gaining attention. Therapy through IN administration has been long practised in the Ayurvedic system of Indian medication and is referred to as "Nasya Karma" (Vyas et al., 2005).

There has been voluminous articles discussing in detail some aspects concerning potential therapeutic applications utilizing this intranasal route for drug delivery to the central nervous system (CNS) however, only several have been successful in formulating a functional dosage form. For nasal route of drug delivery various systems such as nasal spray, nasal pumps, gels, micro-emulsion, suspensions, powders and thermoreversible mucoadhesive gels have been studied (Grassin-Delyle et al., 2012).

Till date, the oral route is much preferred for drug administration as it deems convenient, non-invasive, and cost-friendly. Metformin is an exemplary drug that fits these categories accurately. A study reports an average of 40-41% cost-effectiveness annually for patients consuming Metformin as compared to Acarbose (Gu et al., 2015). However, over time insufficiencies with the oral route such as low solubility of drug and first-pass effect has made it therapeutically less effective (Kaur et al., 2015).

Many drugs struggle to cross the intestinal epithelium into the blood circulation causing it to suffer from poor oral bioavailability (Nidhi et al., 2006). These factors are widely inconsistent and vary between individuals making it even more difficult to determine the effectiveness of the drug in exerting its therapeutic action (Abuhelwa et al., 2017).

Injections are often suggested for patients when rapid therapeutic action is required, precise control of dose and administration rate is called for, for when they are unconscious and cannot be medicated by mouth especially when they can't be assisted by a medical professional (Jin et al., 2015). Depending on the medical status of the patient, the need for rapid action and quick pain relief, brought about the use of injections as a route of drug administration. However, patients are at a great risk of adverse effects such as embolism when the treatment of chronic diseases are tackled through this route (Kaur et al., 2015). Apart from that, self-administration is prohibited as the risk of injuring oneself is very likely. This may lead to potential infection and definite patient discomfort (McDonald et al., 2010).

Due to above factors, the development of alternative or novel drug delivery systems were actively pursued by researchers. Recent studies have shown how the nasal cavity can be exploited for systemic delivery of drugs. This route allows the delivery of small molecular weight drugs, proteins, peptides and even vaccines for rapid onset of action (Illum, 2003). The nasal cavity is a structure that serves to filter and humidify the air we breathe in addition to being abundantly covered with blood vessels. The rich blood supply is formed by the nasal endothelial cells. This thin layer of cells allows for rapid warming of inhaled air as well as rapid absorption of drugs directly into the bloodstream (Hulse et al., 2015), hence systemic action for a drug is possible.

This also allows higher bioavailability and decreased risk of overdose. Drugs administered via this route is not subjected to hepatic or gastrointestinal metabolism offering greater drug availability with a lowered risk of systemic toxicity. This route is becoming highly favourable due to its non-invasive method of administration that minimizes patient discomfort (Rashmin B. Patel et al., 2016). The application of drug utilising nasal delivery have been well demonstrated in treatment of nasal congestion, rhinitis, sinusitis and other allergic-associated diseases. Emergency treatment drugs can be given through the nasal cavity for rapid and localized action.

Recently, researchers focus has shifted more towards nasal applications for systemic drug delivery discovering ways of improving the delivery of drug directly to the central nervous system (CNS) (Shah et al., 2015). It was suggested earlier that the nasal route can also be used for systemic delivery of important drugs such as painkillers, and for centrally-acting drugs such as stimulants and anti-depressants (Illum, 2002). The possibility is due to the presence of olfaction system within nasal cavity. Olfaction is a sensory system that human use to identify food or danger at primitive times. The olfactory cleft is located at the roof of the nasal cavity in near proximity to the cribriform plate (Pinto, 2011). The neuroepithelium of the olfactory region consists of the olfactory neural cells, sustentacular or supporting cells and the basal cells. The olfactory neural cells, or axons, originating at the olfactory bulb are interspaced between supporting cells. They terminate at the apical surface of the olfactory neuroepithelium (Figure 1.1). This structure is the only gateway to the brain that is exposed to the external environment. Drugs gain access directly from the nasal mucosa to the brain and spinal cord utilizing pathways along olfactory and trigeminal nerves with the aim of treating CNS disorders while minimizing systemic exposure.

However, systemic delivery of therapeutics to the CNS is not effective due to the blood brain barrier (BBB) and blood cerebrospinal-fluid barrier (BCBSF) present in the brain (Figure 1.2) (Mittal et al., 2014).

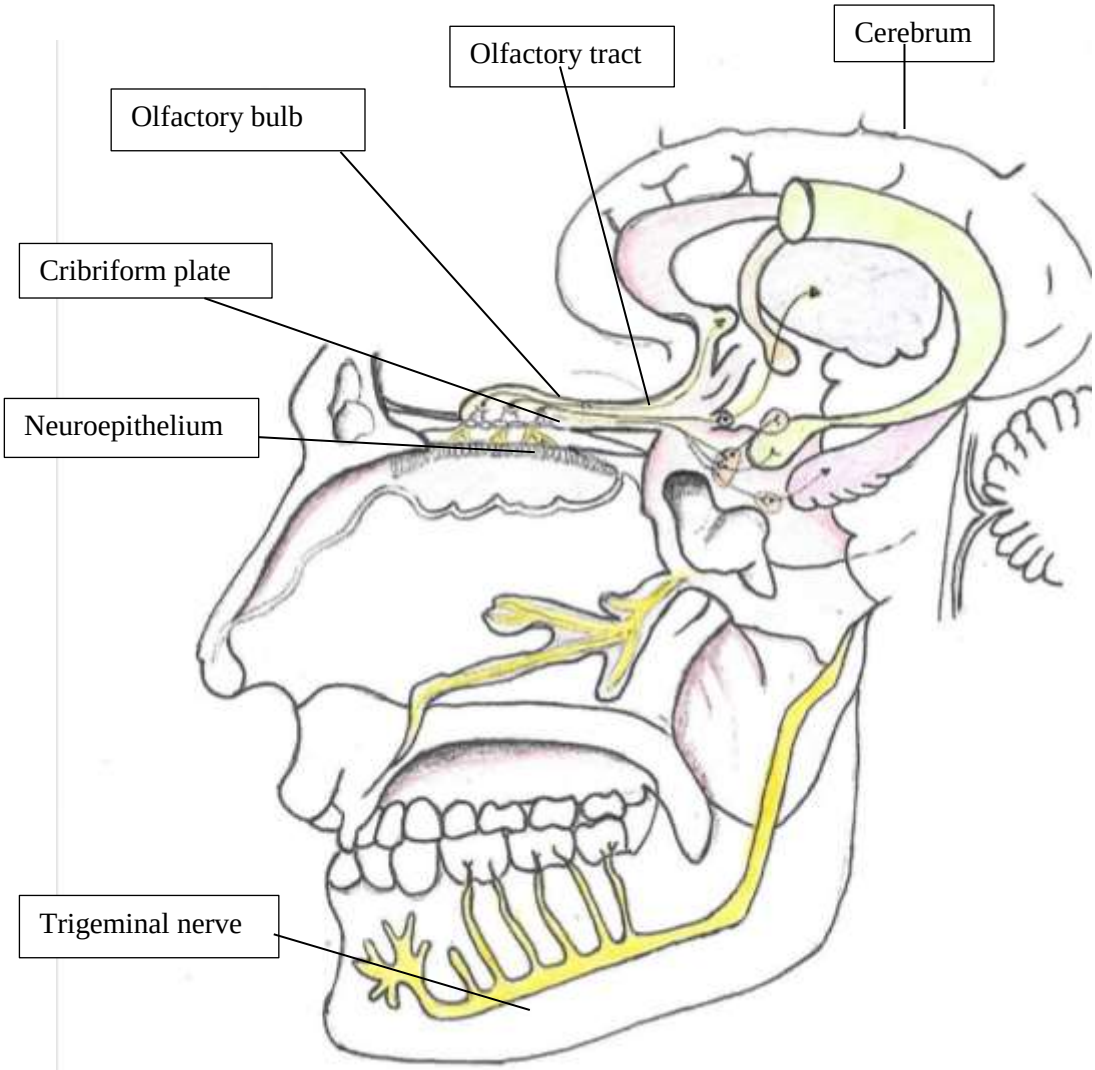

Figure 1.1 Olfactory system showing the olfactory nerves, bulb and tract.

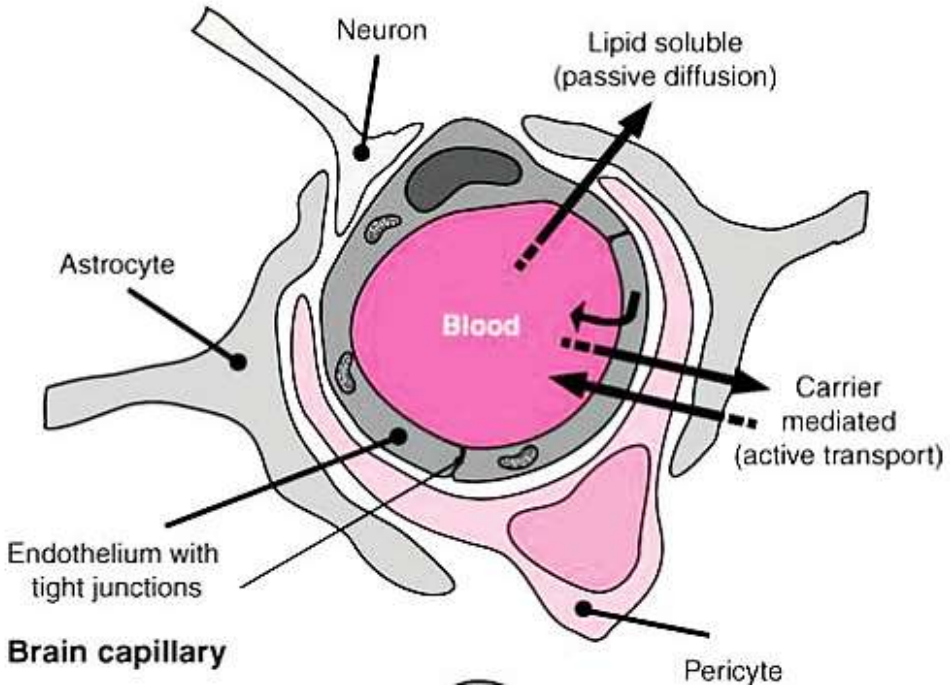

Figure 1.2 Brain capillary in Blood brain barrier (Bodor & Buchwald, 2003).

Any malfunction or breakdown of neuronal function leads to CNS diseases. The nervous system is a complex, intricate system which regulates and coordinates body activities (Krumholz & Ting, 2006). Epilepsy is caused by abnormal firing of neurons and causes recurrent and unprovoked seizures. Groups of cells and neurons in the brain produce impulses that control body movements, thoughts and sensations. When these impulses occur excessively, a seizure is produced. Patients experiences an “aura” or a warning before it takes place. This is usually followed by a seizure where twitching or jerking movements you can’t control. Patients usually lose awareness and consciousness and sometimes even bowel control (Riviello, 2003). There are several types of seizures and they can either be idiopathic or exhibit epileptic syndromes. The International League against Epilepsy classifies the three main types of seizures: partial, generalized, and unclassified.

The older antiepileptic medications such as carbamazepine (CBZ) and sodium valproate are still being used as a treatment and many studies have been conducted to see their effectiveness against new antiepileptic agents such as gabapentin, oxacarbezepine and lamotrigine, topiramate (Marson et al., 2007). However, these agents are orally delivered to treat seizures. These drugs are still subjected to the first-pass effect and drugs loss before reaching the site of action. Because the brain is tightly segregated from the circulating blood by a unique membranous barrier, the BBB, many pharmaceuticals cannot be efficiently delivered to, or sustained within the brain; hence, they are ineffective in treating cerebral diseases. Therefore, drug delivery methods that can provide brain delivery, or eventually preferential brain delivery (i.e. brain targeting), are of particular interest (Zhang et al., 2012).

The brain, a delicate organ, has to be isolated and tightly protected from blood-borne substances in order to perform its many vital functions. Unfortunately, the mechanisms that prevent intrusive environmental chemicals from accessing the brain also prevent therapeutic chemicals from doing the same. (G. Miller, 2002). Therefore, many pharmaceuticals are ineffective in treating CNS diseases because they cannot be properly delivered to or sustained within the brain (Patabendige et al., 2009). According to the Centers of Disease Control and Preventions (CDC), approximately 3.4 million people in the U.S. suffer from epilepsy (2015 data). While extraordinary breakthroughs have been achieved in the treatment of epilepsy, with over 20 drugs, it poses disappointing results with up to one-third of the patients still enduring active seizures or unbearable side-effects related to the medications. Certainly, pharmaco-resistance could most likely be one of the major underlying causes in the failure of the anticonvulsant therapy (Serralheiro et al., 2015). Hence, a growing interest on exploring intranasal administration as means to deliver therapeutics to the brain had started gaining interest.

1.2 STATEMENT OF PROBLEM

The effectiveness of many CNS acting drugs are severely reduced because of the BBB restriction and problematic to deliver at an exact measurable dose (Bodor & Buchwald, 2003). Solution-based formulation application onto the nasal mucosa would be troublesome and inconvenient due to the rapid nasal clearance of liquid formulation. Consequently, these cause a rapid elimination of CNS drug such as carbamazepine from the nasal cavity by mucociliary beating (a clearance rate of 15 min). It is also difficult to design a viable intranasal formulation (Pardeshi & Belgamwar, 2013).

To deal with the above problems, our approach was to develop a thermoreversible sol-to-gel system, where it remains a solution in 2-4 °C and converts to gel when applied to nasal cavity. The present study is designed to make up for voids such as rapid mucociliary clearance even before proper absorption of the drug can take place. Development of a thermoreversible system would help improve the bioavailability of the drug from the site of action. The nasal cavity has been selected due to ease in accessibility and highly vascularized tissue area.