เภสัชจลนศาสตร์ของยาไดอาซีแพมผ่านกระพุ้งแก้มและไส้ตรงเพื่อการระงับชักในเด็ก

นายเด่นพงศ์ พัฒนเศรษฐานนท์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาเภสัชศาสตรมหาบัณฑิต
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BUCCAL AND RECTAL PHARMACOKINETICS OF DIAZEPAM FOR TREATMENT OF SEIZURES IN CHILDREN

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นายเด่นพงศ์ พัฒนเศรษฐานนท์ : เภสัชจลนศาสตร์ของยาไดอาซีแพมผ่านกระพุ้งแก้มและไส้ตรงเพื่อการระงับชักในเด็ก. (BUCCAL AND RECTAL PHARMACOKINETICS OF DIAZEPAM FOR TREATMENT OF SEIZURES IN CHILDREN) อ. ที่ปรึกษา : รศ.ดร.ดวงจิต พนมวัน ณ อยุธยา, อ. ที่ปรึกษาร่วม : พ.ญ. สุรภี เรืองสุวรรณ : 99 หน้า. ISBN 974-03-1021-4

การศึกษานี้มีวัตถุประสงค์เพื่อศึกษาถึงเภสัชจลนศาสตร์ของยาไดอาซีแพมเมื่อให้ยาทางกระพุ้งแก้มและศึกษาความเป็นไปได้ที่จะใช้เป็นวิถีใหม่ในการให้ยาเพื่อระงับอาการชักในเด็กแทนการให้ยาทางทวารหนัก โดยเปรียบเทียบพารามิเตอร์ทางเภสัชจลนศาสตร์ระหว่างการให้ยาทางกระพุ้งแก้มกับทางทวารหนัก การศึกษานี้เป็นแบบ Opened-label, randomized, 2-way crossover trial โดยทำการศึกษาในผู้ป่วยเด็กโรคลมชัก 20 ราย ณ สถาบันสุขภาพเด็กแห่งชาติมหาราชินี

ผู้ป่วยที่เข้าร่วมการศึกษามีอายุระหว่าง 3-13 ปี น้ำหนักอยู่ระหว่าง 12 ถึง 79 กิโลกรัม มี 12 รายที่เป็นเด็กผู้หญิง ขนาดยาที่ได้รับอยู่ระหว่าง 0.13-0.5 มิลลิกรัม/กิโลกรัม ผู้ป่วยทุกรายมีการทำงานของตับและไตปกติ ค่าเฉลี่ยระดับยาสูงสุดในพลาสมาของผู้ป่วยแต่ละราย (C_{max}) เท่ากับ 264.07 $\pm$ 149.53 และ 314.84$\pm$180.33 นาโนกรัม/มิลลิลิตร หลังจากได้รับยาทางกระพุ้งแก้มและทางทวารหนักตามลำดับ โดยค่าเฉลี่ยดังกล่าวไม่แตกต่างกันอย่างมีนัยสำคัญทางสถิติ (P=0.184) เมื่อพิจารณาสัดส่วน C_{max} ที่ช่วงความเชื่อมั่น 90% พบว่า C_{max} ของการให้ยาทางกระพุ้งแก้มอยู่ในช่วง 63% ถึง 104% ของการให้ยาทางทวารหนัก เวลาที่ระดับยาขึ้นสูงสุด (T_{max}) หลังจากให้ยาทางกระพุ้งแก้มช้ากว่าการให้ยาทางทวารหนักอย่างมีนัยสำคัญทางสถิติ (15.75 $\pm$ 7.83 และ 11.5 $\pm$ 5.64 นาที; P=0.031) ค่าคงที่ของการดูดซึมยาทางกระพุ้งแก้มเท่ากับ 21.81 $\pm$ 35.40 ต่อชั่วโมง ขณะที่ทางทวารหนักเท่ากับ 51.64 $\pm$ 76.91 ต่อชั่วโมงซึ่งไม่แตกต่างกันทางสถิติ (P=0.153) หลังจากการให้ยาไม่พบอาการข้างเคียงเกี่ยวกับระบบไหลเวียนเลือดและระบบทางเดินหายใจ ประสิทธิผลทางคลินิกในการระงับการชักไม่สามารถประเมินได้ในการศึกษานี้ อย่างไรก็ตาม พบว่ามีผู้ป่วย 2 รายในแต่ละกลุ่มที่ C_{max} ถึง 500 นาโนกรัม/มิลลิลิตร ซึ่งเป็นระดับยาที่เชื่อว่าสามารถระงับอาการชักได้ ภายใน 5 นาทีหลังได้รับยา ผู้ป่วย 14 รายที่ได้รับยาทางกระพุ้งแก้ม และ 15 รายที่ได้รับยาทางทวารหนักมี C_{max} สูงถึงระดับที่ควบคุมอาการชักได้ (200 นาโนกรัม/มิลลิลิตร) ภายใน 15 นาทีหลังได้รับยา มีความเป็นไปได้ที่การให้ยาไดอาซีแพมทางกระพุ้งแก้มจะสามารถทดแทนการให้ยาทางทวารหนักได้โดยเฉพาะอย่างยิ่งถ้ามีการปรับปรุงสูตรตำรับให้เหมาะสมกับการให้ยาทางกระพุ้งแก้มยิ่งขึ้น อย่างไรก็ตามควรมีการศึกษาเพิ่มเติมเกี่ยวกับการให้ยาในขนาดที่สูงขึ้นในผู้ป่วยขณะชัก เพื่อศึกษาผลตอบสนองแท้จริงทางคลินิกต่อไป

ภาควิชา เภสัชกรรม ลายมือชื่อนิสิต.....

สาขาวิชา เภสัชกรรมโรงพยาบาลและคลินิก ลายมือชื่ออาจารย์ที่ปรึกษา.....

ปีการศึกษา 2544 ลายมือชื่ออาจารย์ที่ปรึกษาร่วม.....

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DENPONG PATANASETHANONT : THESIS TITLE. BUCCAL AND RECTAL PHARMACOKINETICS OF DIAZEPAM FOR TREATMENT OF SEIZURES IN CHILDREN THESIS ADVISOR : ASSOC. PROF. DUANGCHIT PANOMVANA NA AYUDHYA, Ph.D. THESIS COADVISOR : SURAPEE RUANGSUWAN, M.D., 99 pp. ISBN 974-03-1021-4

The purpose of this study were to examine the pharmacokinetics of diazepam administered via buccal route and feasibility of buccal administration to be an alternative to rectal administration for treatment of seizure in children by compared the pharmacokinetic parameters after buccal and rectal administrations. The opened-label, randomized, 2-way crossover trial was carried in twenty epileptic children at Queen Sirikit National Institute of Child Health.

Twelve of the twenty epileptic children were female, the age range was 3-13 years and the weight range was 12 to 79 kg. The dose received varied between 0.13-0.5 mg/kg. All of them were normal in renal and hepatic functions. Mean of the C_{max} when observed for individual subjects was 264.07 ± 149.53 ng/mL after buccal administration while appeared to be 314.84 ± 180.33 ng/mL after rectal administration. There were no significant differences between the two routes of administration (P=0.184). 90% confident interval of C_{max} ratio showed that C_{max} after buccal administration was between 63% to 104% of rectal diazepam administration. Mean of time to reach C_{max} (T_{max}) after buccal administration was longer than after rectal route significantly (15.75 ± 7.83 and 11.5 ± 5.64 minutes, P=0.031). Absorption rate constant (K_a) was 21.81 ± 35.40 $hour^{-1}$ for buccal route and 51.64 ± 76.91 $hour^{-1}$ for rectal route with no statistical different between route of administration (P=0.153). No cardiovascular and respiratory toxicity was recorded in this study and the clinical efficacy could not be evaluated. There were two out of twenty patients after each route whose C_{max} reached the target concentration for termination of seizure (500 ng/mL), both patients after buccal route and one patients after rectal route reached the target level within 5 minutes. There were 14 patients after buccal administration and 15 patients after rectal administration whose C_{max} reached the level necessary to control seizures (200 ng/mL), two patients after buccal route and five patients after rectal route reached the 200 ng/mL level within 5 minutes. Further study with higher dosage at the time of seizure should be performed to observe the true effects on clinical outcome. It seems feasible for buccal route to be use as an alternative to rectal route for diazepam administration in active seizure children especially after a better buccal formulation has been developed.

Department Pharmacy Student's signature…….

Field of study Hospital and clinical pharmacy Advisor's signature……

Academic year 2001 Co-advisor's signature……...

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A number of individuals contributed towards much of this work. I would like to take this opportunity to thanks for contribution.

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ABBREVIATION

ALT	=	Alanine aminotransferase
AUC	=	Area under the concentration-time curves
BD	=	Buccal diazepam administration
BUN	=	Blood urea nitrogen
Cl	=	Clearance
C_{max}	=	Maximum plasma drug concentration or peak plasma concentration
Cr	=	Creatinine
IS	=	Internal standard
K_a	=	Absorption rate constant
K_e	=	Elimination rate constant
RD	=	Rectal diazepam administration
T_{max}	=	Time to reach maximum concentration (C_{max})
$T_{1/2}$	=	Half-life
$T_{1/2\alpha}$	=	Distribution half-life
$T_{1/2\beta}$	=	Elimination half-life
V_d	=	Volume of distribution

CHAPTER I

INTRODUCTION

Seizures are a common neurologic disorder in the pediatric age group and occur with a frequency of 4-6 cases/1,000 children. They are the most common cause for referral to a pediatric neurology practice.[1] Treatment should be initiated early in patients who are prone to seizure cluster or prolonged partial seizures that may generalize or progress to status epilepticus.

Seizures in children usually cease spontaneously within 5-10 minutes after they started and are rarely associated with significant sequelae. The chance of a seizure to stop spontaneously decreases significantly after 5-10 minutes. Similarly, the efficacy of anticonvulsant decreases after 10-15 minutes of consuming while the risk of adverse effects increase. Convulsive seizures lasting longer than 30 minutes constitute status epilepticus and may be complicated by cardiorespiratory depression and brain injury. There is increasing evidence that the longer seizures persist, the more difficult they are to stop.

Since convulsive status epilepticus is the most common neurological medical emergency and continues to be associated with significant morbidity and mortality[3-5] , if prompt prehospital treatment is given, fewer antiepileptic drugs (AEDs) are required in the emergency department and seizures tend to be shorter, result in decreasing of morbidity and motality.[6-8] So, early treatment before admission to hospital is best with an effective medication that can be administered safely.

Recently there have been attempt to abort status epilepticus by treating prolonged or repetitive seizures with benzodiazepines. Rectal diazepam has been used successfully in the treatment of acute episodes[2,9-12] and is widely accepted for its safety, particularly in children.

The rectal cavity provides an excellent absorptive surface for the absorption of lipophilic drugs and high blood concentrations of diazepam can be achieved within minutes. The drug had been shown quite effective in aborting prolonged seizure or eliminating cluster of seizures.[9] However, rectal administration of drugs in an emergency is very difficult and not always acceptable or convenience. When an older child or adult is having a generalized tonic-clonic seizure, a caregiver or parents may find that it is very inconvenient to remove the necessary clothes; bend the knees, and introduce the tip of the syringe into the rectal cavity without another pair of hands. Rectal administration of a drug in the public is also problematic. An effective treatment that can easily be administered by a more convenience route is therefore needed.

Since the mouth and rectum have similar surface areas and pH, are rich in blood supplies, and absorption is directly into the systemic circulation, which avoid high first-pass metabolism[13], buccal diazepam may offer a suitable alternative to rectal diazepam in treatment of acute seizures and lead the way to study and develop a more suitable commercial formulation.

Nowadays, there are still lack of pharmacokinetic data of diazepam administered by buccal route including the clinical outcome of buccal diazepam when use in the treatment of acute seizure.

Therefore, this study was designed to compare the pharmacokinetics of diazepam after administration through rectal and buccal routes in order to determine whether or not buccal route can be use as an alternative to rectal route for treatment of acute seizure with diazepam in epileptic children.

Objectives

1. To study the pharmacokinetics of diazepam administered by buccal route compared with rectal route in epileptic children.
2. To study the feasibility of using buccal route as an alternative to rectal route for treatment of seizure with diazepam in children.

Significance of the Study

1. This study will provide information on the pharmacokinetic parameters of diazepam via buccal and rectal route in Thai children, which can be used to calculate the optimum doses for Thai children.
2. This study will provide information on the clinical outcome and the pharmacokinetic parameters of diazepam compared between rectal and buccal routes.
3. This study will provide information on the possibility of using buccal route as an alternative to rectal route for treatment of seizure with diazepam in children.

CHAPTER II

REVIEW OF LITERATURE

1. Seizure in Childhood [1, 3, 14-18]

Seizures are a common neurologic disorder in the pediatric age group and occur with a frequency of 4-6 cases/1,000 children. They are most common cause for referral to a pediatric neurology practice. The presence of a seizure disorder does not constitute a diagnosis but is a symptom of an underlying central nervous system (CNS) disorder that required a thorough investigation and management plan.[1]

A seizure is defined as a paroxysmal involuntary disturbance of brain function that may manifest as an impairment or loss of consciousness, abnormal motor activity, behavioral abnormalities, sensory disturbances, or autonomic dysfunction. Some seizures are characterized by abnormal movements without loss of impairment of consciousness. Epilepsy is defined as recurrent seizures unrelated to fever or to an acute cerebral insult.

Status epilepticus has been defined by the International League Against Epilepsy and the World Health Organization as a "condition characterized by an epileptic seizure that is so frequently repeated or so prolonged as to create a fixed and lasting condition". Currently it is accepted by most clinicians that a seizure lasting 30 minutes or longer constitutes status epilepticus.

Status epilepticus often is a manifestation of a serious cerebral insult and warrants a full diagnosis evaluation. It occurs as an idiopathic event in less than 10% of affected children. Febrile seizures are a common cause of status epilepticus in children younger that 6 years of age. Abrupt drug withdrawal (poor compliance) is one of the most common causes of status epilepticus in patients with known epilepsy.

Intercurrent infections and sleep deprivation may also precipitate status epilepticus in epileptic children.

Status epilepticus can be classified following the current international classification of seizures. The most common form of status epilepticus is generalized convulsive, which is characterized by generalized tonic-clonic or clonic seizure activity. Cerebral damage during status epilepticus may be cause by direct and indirect causes. Direct damage may be caused by the seizure activity itself. Neuronal injury may also result from indirect systemic changes secondary to status epilepticus.

Systemic Complications [14]

Hyperthermia

This complication may occur during generalized convulsive status epilepticus as a result of sustained motor activity. Hyperthermia increases the body's metabolic demands and need for oxygen, and also contributes to rhabdomyolysis and subsequent myoglobinuria.

Cardiovascular Changes

Increased catecholamine serum concentrations during status epilepticus result in prominent vasoconstriction. The increase in pulmonary and systemic vascular resistance causes systemic, pulmonary, and left atrial hypertension and is probably responsible for the 50% drop in cardiac output that is observed during the initial 5-10 minutes of a status epilepticus episode. Pulmonary arterial and left atrial blood pressures return to normal after 15-30 minutes and systemic arterial blood pressure after 60 minutes. If status epilepticus continues, cardiac failure may occur with a fall in cardiac output and a drop in systemic blood pressure. Hypotension results in reduction of cerebral blood flow, which depends on a systemic blood pressure. Decreased cerebral blood flow in the presence of an increased neuronal metabolic rate may result in cellular death and permanent neurologic sequelae. Cardiac arrhythmia

may also occur during status epilepticus. This mechanism may be implicated in the sudden unexplained deaths of some epileptic patients.

Hypoxia

Respiratory disturbances accompany cardiovascular changes. Hypoventilation, apnea, hyperventilation, Cheyne-Stokes respiration, increased oral-bronchial secretions, aspiration of stomach contents, and pulmonary congestion can all occur during status epilepticus and result in systemic and cerebral hypoxia. The respiratory dysfunction may be aggravated by the administration of antiepileptic drugs in an effort to control seizure. The hippocampus and cerebellum are particularly susceptible to hypoxia.

Acidosis

Severe metabolic acidosis causes by an excessive production of lactic acid in muscle is observed in convulsive status epilepticus. Lactate is rapidly metabolized after cessation of the seizure, and the acidosis resolves in 1 hour. The Frequently a variable respiratory contribution to the acidosis is observed. The risk of brain damage during status epilepticus is dependent of the degree of lactic acidosis in the absence of cardiac failure. Thus correction of metabolic acidosis in status epilepticus is rarely necessary.

Hypoglycemia

Hypoglycemia occurs initially during status epilepticus because of high epinephrine levels. However, hypoglycemia occurs approximately30 minutes after the onset of status epilepticus. The hypoglycemia may add to the mismatch of decreased energy substrate in the face of increased neuronal metabolic demand.

Hyperkalemia

Hyperkalemia may occur during convulsive status epilepticus due to muscle necrosis. However, it may also complicate nonconvulsive status epilepticus since it

has been observed in paralyzed animals, probably due to an alpha-adrenergic mechanism. Hyperkalemia may increase the risk of cardiac arrhythmia.

Myoglobinuria

Increased muscle activity during convulsive status epilepticus, especially if associated with hyperthermia, may cause rhabdomyolysis with subsequent myoglobinuria that may produce renal failure.

Cerebral Blood Flow and Intracranial Pressure

The elevation of the mean systemic arterial blood pressure causes an increase in the cerebral perfusion pressure, which in combination with a paralyzed cerebral autoregulation produces and increase in cerebral blood flow of 200% to 600% above normal. The increased cerebral perfusion pressure persists for 40 minutes and subsequently returns to normal even if seizure activity continues and catecholamine concentrations remain high. Despite the significant increase in cerebral blood flow, there is only a brief, slight rise of 7 to 20 mm Hg in the ICP. By the end of the first 15 minutes of seizure activity, the ICP returns to normal. However, cerebral edema occurs in some cases.

Pulmonary edema

Repetitive seizures rather than a prolonged single seizure may produce pulmonary edema. It has been found in at least one third of patients who died during status epilepticus. The pulmonary edema seen in status epilepticus is neurogenic in origin since it does not occur in experimental animals after cervical cord transection and it is independent of hypoxia and airway obstruction.

Currently mortality due to status epilepticus is primarily attributable to underlying diseases. Previously a 10% to 30% mortality rate was reported. Recently it has been found that children with status epilepticus lasting 30 minutes or longer have a mortality rate of 3%-6%. Perhaps this decrease is a consequence of more rapid diagnosis and improved management.

Management of Acute Seizure and Status Epilepticus.[14-18, 6-7, 19-20]

Since habitual convulsive seizures last less than a few minutes in most patients, a suggested operational definition of status epilepticus is either continuous seizures lasting at least 5 minutes or two or more discrete seizures not separated by complete recovery of consciousness. There is increasing evidence that the longer seizures persist, the more difficult they are to stop.

Any seizure during childhood, except those of short duration (2 to 5 minutes) should be treated quickly and effectively. The parents–administered emergency treatment is especially important if transit time to the physician or hospital is more than 15 minutes. Rectal diazepam in solution (an ordinary intravenous preparation, containing 5 mg diazepam per milliliters) is very suitable for acute treatment, as its administration is simple and quick, the absorption fast and complete, and anticonvulsant plasma concentrations of diazepam are attained in 2 to 4 minutes.[7] All known commercial intravenous diazepam preparations are applicable for rectal administration, either in specially made pre-filled rectal tubes of plastic (eg. Dumex Stesolid Rectal Tubes^{R}) or with an ordinary 2 mL plastic syringe fitted with 4-5 cm soft plastic tip. The treatment should be administered with the child lying on their side or prone, to secure high bioavailability and anticonvulsant effect. To avoid discharge of the drug the child's buttock should be squeezed together for 3 to 5 minutes after administration.

Although intravenous diazepam is the treatment of choice during a seizure, rectal diazepam in solution is a rational alternative and the only "non-professional intravenous line". The treatment is simple, quick and effective, and has a few major side effects.[21-24]

The majority of seizures last a short time and cease spontaneously or less than 5 minutes after rectal diazepam. The administration of 2 doses of diazepam at home at 5-minute intervals is justified in the event of continued convulsions, i.e. a maximum of diazepam 1 mg/kg in total, as a risk of respiratory depression is extremely slight. In general, intravenous diazepam should only be given

supplementary to rectal diazepam if intubation is possible. Seizures are diazepam-resistant if the total dose of 2 to 3 mg/kg of diazepam (rectal plus intravenous) has not resulted in remission in 30 to 60 minutes.

Most generalized convulsive status epilepticus episodes represent a medical emergency. Assessment and therapy are performed simultaneously in patients with status epilepticus. The management of the child necessitates a team approach within an emergency department. Antiepileptic drugs are essential for treating status epilepticus, and the systemic consequences must be recognized and treated. The primary goals of treatment are to support vital functions, control seizure activity, and identify and treateologic and precipitating factors. The following steps need to be followed to properly assess and manage the patient.

Step 1: Stabilize the Patient (0 to 15 minutes)

1. Protect the patient from injury but do not restrain because this may cause fractures or soft tissue injuries.
2. Place the patient in slight Trendelenburg position with the head turned to one side to facilitate drainage of oral secretions or vomit and prevent aspiration.
3. Assess and monitor cardiorespiratory function, including respiratory rate, type of respiration, presence of cyanosis, SaO_2, systemic arterial blood pressure, and ECG finding.
4. Secure the airway and support respiration if necessary.
5. Monitor body temperature.
6. Identify the type of seizure activity (generalized vs. partial, convulsive vs. nonconvulsive, etc.) since this will have both prognostic and therapeutic implications and monitor duration of seizure activity.
7. Take a short history, and perform a general and neurologic examination to seek any clues that might indicate and etiology for the status (i.e., Med-Alert bracelet, head injury, infection, etc.)
8. Establish IV access. Two lines are preferred, one with 0.95 sodium chloride solution if drug such as phenytoin are used and the second to administer substances such as glucose and pyridoxine. However, placing and IV line is not always easy, especially in small children. If only one IV line is used, do not mix

phenytoin with dextrose because the phenytoin may crystallize, reducing its efficacy.

9. Obtain a blood sample for laboratory tests as the IV line is established. Send blood for complete blood count, electrolytes, calcium, magnesium, glucose, BUN, creatinine, antiepileptic drug levels, arterial blood gas, and toxicology screen. Because status may cause hypoglycemia and hyponatremia, it is important to continue to monitor serum glucose and electrolytes.
10. Administer IV 50% glucose, 1 to 2 mL/kg (or 2 to 4 mL/kg of 25% glucose). Thiamine, 100 mg IV, is given before the glucose administration in patients suspected of alcoholism.
11. If the patient is infant, administer pyridoxine, 100 mg IV.
12. Treat hypotension if present.
13. Control hyperthermia.

Step 2: Control Seizure Activity (10 to 20 minutes)

Table 1 shows the recommended dose and calculations associated with the most commonly used drugs for status epilepticus. Remember that the most common mistakes made in treating status epilepticus are administration of an inadequate dose and use of a short-acting agent alone (i.e., diazepam).

1. Start with IV lorazepam (or diazepam if lorazepam is not available).
2. Concomitant with the benzodiazepine infuse 20 mg/kg phenytoin in 0.9% sodium chloride solution at the rate no faster than 1 mg/kg/min while monitoring ECG and blood pressure.
3. In newborns and infants use IV phenobarbital instead of phenytoin at the dose of 20 mg/kg.

Most patients' clinical seizures will be controlled with this treatment. However, depending on the etiology, clinical seizures may be refractory and continue despite this standard treatment.

Table 1 Drugs used in the treatment of status epilepticus.[14]

Medication	Route	Dose	Infusion rate	Caution
Diazepam	IV	0.2-0.4 mg/kg (max dose: 5 mg < 5 yr; 10 mg > 5 yr)	1-2 mg/min	Respiratory depression
	Rectal	0.5-0.75 mg/kg		
Lorazepam	IV	0.05 mg/kg (may repeat x 3)	1 mg/min	Respiratory depression
Midazolam	IV	0.15 mg/kg bolus followed by 1.0 μg/kg/min infusion		Respiratory depression
Phenytoin	IV	20 mg/kg (dilute in 0.9% sodium chloride solution	1 mg/kg/min	Hypotension, cardiac conduction defects
Phenobarbital	IV	20 mg/kg	2 mg/kg/min	Respiratory depression

Step 3: Follow-up after Stabilization and Seizure Control

1. Obtain bacterial and viral cultures of blood, nasopharyngeal secretions, and CSF if clinically indicated. A complete septic work up must be done in neonates with status epilepticus.
2. Request an EEG. This is especially important if it is questionable whether seizures occurred, in nonconvulsive status epilepticus, and in unexplained hemiparesis or paralysis.
3. Continuous monitoring of vital signs and frequent assessment of neurologic status must be performed during and after status epilepticus is controlled and until the patient becomes stable.

2. Principle of Drug Absorption by Sublingual and Rectal Routes

Rectal Drug Absorption[13, 27]

Rectal administration of drugs has been used since ancient times to produce local effects. In addition, the rectal route may be used for systemic administration of drug for the following reasons

a. The presence of nausea and vomiting, when the patient is unconscious.
b. The presence of disease of the upper gastrointestinal tract which affects absorption of drug given orally.
c. An objectionable taste (a factor which may be particularly important in children.
d. The achievement of a rapid systemic effect by giving a drug in suitable solution (as an alternative to parenteral administration).
e. Drug absorption may be easily discontinued in the event of an accidental overdose.
f. The rate of drug absorption is not influenced by ingestion of food or the rate if gastric emptying.
g. Firs-pass elimination of high clearance drugs may be partly avoided.
h. Contact with digestive fluids of the upper gastrointestinal tract is avoided, thereby preventing breakdown of some drugs.

The disadvantages associated with administration of drug rectally include:

a. Interruption of absorption by defecation, which may occur particularly with irritant drugs.
b. The surface area of the rectum is far smaller for absorption than that of the duodenum.
c. The fluid contents of the rectum are much smaller than those of the duodenum and this may produce problems with dissolution of some drugs.
d. Degradation of some drugs by microorganisms may occur in the rectum.
e. Patient acceptability may be a problem, at least in some countries.

The length of the human rectum is approximately 10-15 cm and circumference 15-35 cm. Its fluids contents have pH of 7-8. Because villi are absent, the surface area is small (200-400 cm^2) compared with that small intestine: (200 m^2). The rectum is drained by three veins: superior, middle and inferior rectal veins. The inferior and middle rectal veins drain the lower part of the rectum, while the superior rectal vein drains the upper part. Between these three veins exist extensive anastomoses.

Two factors are of major importance with respect to the venous drainage of the rectum and consequently the transport of absorbed drug into the systemic circulation: site of the absorption and direction blood flow. If the drug is absorbed in the upper part of rectum, it is transported directly to the portal system and passes through the liver while, following absorption in the lower rectum, the drug is transported directly to the systemic circulation. In general, this implied that hepatic first–pass elimination is avoided when a drug is administered in the lower part of the rectum. However, a complicating factor is that there is no precise anatomical division between the area draining to the portal and that draining to the systemic circulation, because of the presence of anastamoses.

Buccal and Sublingual Administration[13, 25-28]

Although the total buccal and sublingual surface area is small (200 cm^2) and has a pH of 6.2-7.4 the potential exists for rapid absorption of drugs since these areas are rich in blood and lymphatic vessels; thus rapid systemic action may achieved by administering drugs sublingually or buccally. Only potent drugs are likely to be effective when administered in this way. Although emphasis has been placed on the structural differences between keratinized and non-keratinized oral mucosae, there is no compelling evidence for major differences between them in drug absorption capabilities. Studies using peroxidase and lanthanum have shown equivalent impermeability in keratinized and non-keratinized oral epithelia. The conclusion from these studies, and others using electrolytes, was that rather than the keratinized surface layer representing a barrier, it was the presence of membrane-coating granules that was important. In addition, intercellular junctions did not seem to influence permeability whereas the intercellular space itself, although only a minor (1%)

component of tissue volume, could do so. The ingress of electrolytes and lipid-soluble substances by this pathway suggests that superficial layers could be traversed.

In certain circumstances there may be differences in permeability between keratinized and non-keratinized oral epithelium, as increased permeability of non-keratinized oral epithelium to water has been reported. Other factors that may be important include mucosal disease or thinning of the oral epithelium. Certainly, the sublingual epithelium is slightly thinner than buccal epithelium, and its intermission in saliva would tend to give it an increased permeability to most substances.

An important advantage of these routes is that the drug passes directly into the systemic circulation. Thus, hepatic high-clearance drugs, or drugs which are subject to presystemic gut-wall metabolism or decomposition in the gastrointestinal tract, or both, will exhibit higher systemic availability following sublingual, compared with oral, administration.

An important disadvantage of these routes is the maintenance of the drug in the buccal and sublingual area. This varies between subjects and is dependent upon the drug formulation, disintegration, flow of saliva and the rate of drug absorption.

Absorption is highly dependent on the residence time of the drug in the sublingual and buccal area and this may very considerably. In addition, bad tastes or irritation caused by the drug may lead to voluntary expulsion or swallowing. The residence time of the drug used is dependent upon the formulation: a solution has shorter residence time than a tablet. Saliva flow is important since it affects the rate of dissolution of the drug and, if the saliva flow is considerable, there is an increased likelihood that part of the drug will be swallowed before absorption. Therefore the patient must learn to adapt to these routes of administration in order to avoid sucking on the tablet, swallowing the drug before absorption and excessive salivation by the presence of drug in the mouth.

The physicochemical mechanisms involved in the transfer of drug across the oral mucus membrane are similar to those at others cell membranes. Important factors related to the drug are molecular size and shape, solubility at the site of absorption,

degree of ionization and lipid solubility. Drug can cross the mucous membrane either by passive processes or by active processes.

Simple Diffusion: Diffusion through a lipid phase is the major method by which substances transfer across the oral mucosa. The absorption pathway is based on the random motion of molecules from a zone of high concentration to one of low concentration. At first there is rapid passage but this gradually diminishes and rate of penetration is directly proportional to the concentration of the substance placed on the mucosa.

Intercellular movement: Depending on the nature of cell-cell junctions, epithelia have been described as either 'tight' or 'leaky'. The oral epithelium has a low population of tight junctions and could be regarded as leaky, and therefore is likely to allow passage of substances through intercellular spaces. The basal laminar is probably the limiting factor and restricts passage of molecules with a molecular weight >70,000.

Endocytosis: The absorption of solid particles (phagocytposis) or of fluids (pinocytosis) is referred to collectively as endocytosis. Although all of oral mucosa are able to absorb substances by endocytosis it is likely that this mechanism has only a minor role in drug transport from the oral cavity.

Active transport: Metabolic energy is required to transport molecules or ions against a concentration or electrochemical gradient. Although it has been shown that this mechanism is involved in intestinal transport, it is unlikely that it is involved in the mouth.

In general, polar drugs are badly absorbed. Drugs with a moderate lipophillicity are well absorbed, while drugs with a very high partition coefficient are too water-insoluble to achieve a sufficiently high concentration in salivary fluids. The pH of the buccal area is important for the absorption of acid or alkaline drugs, while membrane storage seems to occur for lipophillic drugs such as propranolol. In addition, binding of drugs to macromolecules in the mouth interferes with drug absorption.

The buccal absorption test is useful tool and easy to perform. It has been shown that this test provides a better indication of the passage of drug through biological membranes than do simple partition or rate of partition between water and organic solvents.

There are two routes of transport of absorbed drug into the systemic circulation. Absorption of drug occurs into capillaries, but also uptake into lymph may be significant, as has been demonstrated by buccal administration of para-aminosalicylic acid.

The sublingual and buccal routes of administration are useful when fast action is desired with potent drugs. In addition, first-pass elimination (gut-lumen, gut wall and hepatic) is avoided. Prolonged residence in the mouth can limit usefulness because of patient intolerance, and for long-term drug administration this route is not convenient when conventional formulation are used.

3. Diazepam: Focus on Treatment of Acute Seizures

Diazepam is a benzodiazepine. Diazepam occurs as an off-white to yellow, practically odorless, crystalline powder. The drug is sparingly soluble in propylene glycol and has solubilities of approximately 3 mg/mL in water and 62.5 mg/mL in alcohol at 25ºC. Diazepam has a pK_a of 3.4. Sodium benzoate and benzoic acid are added to the commercially available injection to adjust pH to 6.2-6.9.[29]

Stability [29]

Diazepam injection should be protected from light and stored at 15-30ºC; freezing should be avoided. The manufacturers state that diazepam injection should not be mixed with other drugs or IV fluids. Although some studies indicate that diazepam injection may be compatible with various drugs and IV fluids (e.g., diluted to a concentration of 5 mg/50mL to 5 mg/100mL with 0.9 sodium chloride, 5% dextrose, Ringer's, or lactated Ringer's injection), compatibility may depend on several factors (e.g., the concentration of the drugs, resulting pH, temperature).

Specialized references should be consulted for more specific compatibility information. The addition of diazepam injection to an IV infusion solution or plastic syringes may result in adsorption of diazepam to the plastic container and tubing.[22]

Pharmacologic Properties of Benzodiazepines [30-33]

The effect of the benzodiazepines virtually all result from actions of these drugs on CNS. The most prominent of these effects are sedation, hypnosis, decreased anxiety, muscle relaxation, anterograde amnesia, and anticonvulsant activity. Only two effects of these drugs appear to result from actions on peripheral tissues: coronary vasodilation, seen after intravenous administration of therapeutic doses of certain benzodiazepines, and neuromuscular blockade, seen only with very high doses.

A variety of benzodiazepine-like effects have been observed in vivo and in vitro and have been classified as full agonistic effects (i.e., faithfully mimicking agents such as diazepam with relatively low fractional occupancy of binding sites) or partial agonistic effects (i.e., producing less intense maximal effects and/or requiring relatively high fractional occupancy compared to agents such as diazepam). Some compounds produce effects opposite to those of diazepam in the absence of benzodiazepine-like agonists and have been termed inverse agonists; partial inverse agonists also have been recognized. The vast majority of these effects can be reversed or prevented by the benzodiazepine antagonist flumazenil, which implies mediation by one or more subtypes of benzodiazepine binding sites. In addition, representatives from various classes of compounds behave like flumazenil and act only to block effects of agonists or inverse agonists.

Central Nervous System

While the benzodiazepines affects activity at all levels of the neuraxis, some structures are effected to a much greater extent than are others. The benzodiazepines are not general neuronal depressants, as are barbiturates. All of the benzodiazepines have very similar pharmacological profiles. Nevertheless, the drugs differ in

selectivity, and the clinical usefulness or individual benzodiazepines thus varies considerably.

As the dose of benzodiazepines is increased, sedation progresses to hypnosis and then stupor. The clinical literature often refers to the anesthetic effects and used of certain benzodiazepines, but the drugs do not cause a true general anesthesia, since awareness usually persists, and relaxation sufficient to allow surgery cannot be achieved. However, at "preanesthetic" doses, there is amnesia for events subsequent to the administration of the drug; this may create the illusion of previous anesthesia.

The recent discovery of a molecular basis for numerous benzodiazepine receptor subtypes has provided the rationale for attempts to separate the anxiolytic actions of these drugs from their sedative/hypnotic effects. However, distinguishing between these behaviors remains problematic. Measurements of anxiety and sedation are difficult in human beings, and the validity of animal models for anxiety and sedation is uncertain. The existence of multiple benzodiazepine receptors may partially explain the diversity of pharmacological responses in different species.

Molecular Targets for Benzodiazepines Actions in the CNS

The major molecular targets of the benzodiazepines are inhibitory neurotransmitter receptors directly activated by the amino acid, gamma-aminobutyric acid (GABA). The major type of the $GABA_A$ receptor, is an integral membrane chloride channel that mediates most of the rapid, inhibitory neurotransmission in the central nervous system. $GABA_A$ receptors, which have seven membrane-spanning domains and are coupled to their signal transduction mechanism by G proteins, are not altered by benzodiazepines. According to the $GABA_A$ receptor hypothesis for benzodiazepines action, benzodiazepines directly bind to the receptor/ion channel complex and allosterically modulate its activity. Unlikely barbiturates, benzodiazepines do not directly gate $GABA_A$ receptors but require GABA to express their effects. An array of biochemical and functional evidence supports this hypothesis. Radiolabeled benzodiazepine and GABA analogs bind to brain membranes with high (nanomolar) affinity. Benzodiazepines modulate binding to the GABA site, and GABA alters benzodiazepines binding in allosteric fashion. Typical

benzodiazepines agonists increase the amount of chloride current generated by $GABA_A$ receptor activation, potentiating the effects of GABA throughout the nervous system. Moreover, the behavioral and electrophysiological effects of benzodiazepines usually are reduced or prevented by prior treatment with antagonists at the $GABA_A$ receptor, such as bicuculline. Certain benzodiazepine congeners have been discovered that potently and selectively block both high-affinity binding and biological effects elicited by other benzodiazepines. One such agonist, flumazenil, has clinical use for reversing the effects of high doses of benzodiazepines.

The strongest evidence that benzodiazepines act directly on $GABA_A$ receptors comes from molecular cloning of cDNAs encoding subunits of the $GABA_A$ receptor complex. When the appropriate subunits are expressed in heterologous cells, high-affinity benzodiazepine binding sites are produced along with receptor that mediated GABA-activated chloride conductances. The currents measured in these cells are potentiated by benzodiazepines. The properties of the expressed receptors are quite similar to those of $GABA_A$ receptors found in most central neurons.

The conceptual advances brought about by molecular studies have strengthened the hypothesis that benzodiazepines act mainly at $GABA_A$ receptors. Moreover, molecular diversity helps clarify many previous observations that appeared to conflict with this hypothesis. Nonetheless, some observations are difficult to reconcile with the hypothesis that all effects to benzodiazepines are mediated via $GABA_A$ receptors.

Low concentrations of benzodiazepines induce depressant effects on hippocampal neurons that are not blocked by bicuculline or picrotoxin. The induction of sleep in rats by benzodiazepines also is insensitive to bicuculline or picrotoxin but is prevented by flumazenil. At higher concentrations, corresponding to those producing hypnosis and amnesia during preanesthetic medication or those achieved during the treatment of status epilepticus, the actions of the benzodiazepine may involve the participation of a number of other mechanisms. These include inhibition of uptake of adenosine and the resultant potentiation of the actions of this endogenous neuronal depressant, as well as the GABA-independent inhibition of Ca^{2+} currents, Ca^{2+}-dependent release of neurotransmitters, and tetrodotoxin-sensitive Na^{+} channels.

Respiration

Hypnotic doses of benzodiazepines are without effect on respiration in normal subjects. At higher doses, such as those used for preanesthetic medication or endoscopy, benzodiazepines slightly depress alveolar ventilation and cause respiratory acidosis as the result of a decrease in hypoxic rather than hypercapnic drive; these effects are exaggerated in patients with chronic obstructive pulmonary disease (COPD), and alveolar hypoxia and/or CO_2 nacrosis may result. These drugs can cause apnea during anesthesia or when given with opioids, and patients severely intoxicated with benzodiazepines usually require respiratory assistance only when they have also ingested another CNS-depressant drug, most commonly alcohol.

By contrast, hypnotic dose of benzodiazepines may worsen sleep-related breathing disorders by adversely affecting the control of the upper airway muscles or by decreasing the ventilatory response to CO_2. The latter effect may be sufficient to cause hypoventilation and hypoxemia in some patients with sever COPD, although benzodiazepines may improve sleep and sleep structure in some instances. In patients with obstructive sleep apnea (OSA), hypnotic doses of benzodiazepines may decrease muscular tone in upper airway and exaggerate the impact of apneic episodes on alveolar hypoxia, pulmonary hypertension, and cardiac ventricular load. Many physicians consider the presence of OSA to be a contraindication for the use of alcohol or any sedative-hypnotic agent, including a benzodiazepine; caution also should be exercised in patients who snore regularly, because partial airway obstruction may be converted to OSA under the influence of these drugs. In addition, benzodiazepines may promote the appearance of episodes of apnea during REM sleep (associated with decreases in oxygen saturation) in patients recovering from a myocardial infarction; however, the potential impact of these drugs on survival of patients with cardiac disease has not been investigated as yet.

Cardiovascular System

The cardiovascular effects of benzodiazepines are minor in normal subjects, except in severe intoxication; the adverse effects in patients with obstructive sleep disorders or cardiac disease were noted above. In preanesthetic doses, all

benzodiazepine decrease blood pressure and increase heart rate. With midazolam, the effects appear to be secondary to a decrease in peripheral resistance, but with diazepam they are secondary to a decrease in left ventricular work and cardiac output. Diazepam increase coronary blood flow, possibly by an action to increase interstitial concentrations of adenosine and the accumulation of this cardiodepressant metabolite also may explain the negative inotropic effects of the drug. In large doses, midazolam decreases considerably both cerebral blood flow and oxygen assimilation.

Gastrointestinal Tract

Benzodiazepines are though by some gastroenterologists to improve a variety of "anxiety-related" gastrointestinal disorders. There is a paucity of evidence for direct actions. Benzodiazepines partially protect against stress ulcers in rat, and diazepam markedly decreases nocturnal gastric secretion in human being.

Pharmacokinetic-Pharmacodynamic Relationships

Pharmacokinetics describes the time course of drug amount and concentration in body fluids; pharmacodynamics describes the time course and intensity of drug effect. Correlating drug effect with plasma concentrations allows us to know the pharmacokinetics-pharmacodynamic relationship, which is essential in allowing us to determine the optimal therapeutic activity.

Onset of Pharmacological Activity

A fast onset of action is an essential feature for drugs used in the management of acute seizures. All benzodiazepines exhibit a rapid onset of action and enter the cerebral tissues rapidly, which is consistent with their short distribution half-life.[34] After intravenous administration of diazepam 10 mg in patient with status epilepticus, the time for onset of action ranged from immediate effect to 10 minutes (median 2 min).[35]

Duration of Action

Diazepam appears to have a short duration of action as assessed by the time elapsed before recurrence of seizures: a substantial number of patients relapsed within 2 hours of intravenous administration.[36] In an animal model, the highest concentration of the drug in the brain occurred immediately after infusion was completed and fell rapidly, paralleling the plasma concentration.[37] After an intravenous bolus dose of diazepam 0.3 mg/kg, plasma concentrations fell below 200 ng/mL in less than 50 minutes; 200 μg/L is the concentration considered necessary to control status epilepticus in human.[38-40]

Although little data are available to help us to define the effective drug plasma concentration of diazepam, the minimum plasma concentration required to suppress seizures is thought to range between 200 to 600 μg/L in most emergency setting.[38-40]

Concentration-Effect Relationships

Greenblatt et al.[41] used the electroencephalographic (EEG) profile as direct objective assessment of central effects of diazepam. Eleven healthy volunteers received a single 1-minute intravenous infusion of diazepam 0.15 mg/kg. EEG changes (percentage increase in total EEG amplitude occurring in the 13 to 30 Hz frequency range determined using fast Fourier transform) were maximal at the end of the diazepam infusion. The increase in fractional EEG activity over baseline remained until the 5-hour post-infusion time-point for diazepam. The relationship of mean EEG change to mean plasma concentration was fitted to the sigmoid E_{max} model:

$$E = E_{max} C^A/(B+C^A)$$

Where E is the drug effect, Emax is the maximal drug effect, C is the drug plasma concentration, A is the exponent and B is a constant equal to EC_{50}^A. The apparent concentration association associated with an increase in effect corresponding to 50%

of E_{max} (EC_{50}) value for diazepam was 269 ng/mL. Diazepam produced EEG effects of rapid onset.

Buhrer et al.[42] Also used the increase in the EEG voltage, quantified with a periodic wave form analysis, as a measure of the drug effect of diazepam. Three healthy individuals received different single doses, infused at 10 mg/min for diazepam (15,30 or 50 mg). A time lag was observed between plasma concentration and EEG effect, and a plot of effect versus plasma concentration showed a hysteresis loop indicating that the maximum effect was delayed compared with the maximum drug plasma concentration (C_{max}). An effect compartment was modeled and allowed to estimate the first-order equilibration rate constant (k_{e0}) between plasma and the effect compartment. The average plasma-effect site equilibration half-life ($t_{1/2,ke0}$) of diazepam was 1.6 minutes. The plasma concentration producing EC_{50} estimated for steady state was on average 958 ng/mL for diazepam.

Mould et al.[43] used a simple psychometric task, the digit Symbol Substitution Test (DSST), to study the pharmacokinetics and pharmacodynamics of diazepam after single intravenous infusions of diazepam 0.1 or 0.2 mg/kg in 12 healthy individuals. The data were fitted to a semiparametric model and used to calculate $t_{1/2,ke0}$ and EC_{50}. The mean $t_{1/2,ke0}$ for the pooled high and low dose was 1.2 minutes for diazepam, and the mean EC_{50} value was 116 μg/mL.

The rate of entrance of diazepam into the brain was determined by eye movement recorded after intravenous administration of a single dose of diazepam 5 mg in 6 healthy volunteers.[44] The study demonstrated that diazepam crosses blood brain barrier rapidly. The time to E_{max} was 10 minutes after administration.

Some of the above studies demonstrate a relationship between the plasma concentration of diazepam and its pharmacodynamic effect. However, some caution is warranted as the clinical relevance is not clearly established: the effects measured in healthy volunteers may not be impossible on the clinical effects expected in acute seizures. In particular, the EC_{50} is highly variable according to the method used to

assess diazepam response, although the half-life of plasma effect site equilibration appeared similar in the different studies.

Physicochemical Properties

Diazepam crosses the blood-brain barrier to elicit its pharmacologic effect. The transfer of most drugs across the blood-brain barrier occurs by simple diffusion,[45] the rate of which is mainly governed by physicochemical factors according to Fick's law:

$$\text{Rate of diffusion} = D \times \Delta C \times A/d$$

Where D is the diffusion constant of the drug, ΔC is the drug concentration gradient across the membrane, A is the area of exchange and d is the membrane thickness. The diffusion constant is dependent on physicochemical factors and the drug concentration gradient is dependent on the pharmacokinetics of the drug.

Diazepam is a basic compound with a low molecular weight (309). Its pKa is 3.4 and its partition ratio (octanol/buffer at physiological pH) is 309, thus diazepam is virtually undissociated at physiological pH.

The physicochemical properties of benzodiazepines regulate the rate and extent of entry into the brain and CSF. The speed of equilibration between plasma and effect site is described by the equilibration half-life measured by the elimination of the drug from the effect site. The equilibration half-life is determined factor for onset of drug action in the CNS. The equilibration half-life of the unbound drug is primarily depending upon the partition ratio. The lipophillicity predicts rate of equilibration and faster onset of action for diazepam.

Diazepam is extensively protein bound,[29] and the binding is independent of plasma concentration. The unbound concentration of diazepam is 3%. Cerebrospinal fluid (CSF) concentrations of diazepam parallel unbound drug concentrations in plasma as assessed from published data. The CSF concentrations of diazepam were

2% of their plasma concentration at 0.5 hour after an intramuscular dose of diazepam 10 mg in 42 patients.[46]

Pharmacokinetics

Intravenous Route

After intravenous administration, the $t_{1/2\alpha}$ is short for diazepam (1.9 to 13.3 minutes). The volume of distribution is large, suggesting extensive tissue binding. Diazepam is metabolized in the liver mainly via cytochrome P450 (CYP)-dependent microsomal enzymes. Diazepam is *N*-desmethylated into *N*-desmethyldiazepam, which has an anticonvulsant activity of about one-third the potency of the parent drug. This metabolic pathway is dependent on CYP2C9 and CYP3A. Diazepam clearance is 0.038 ± 0.015 L/hr/kg and the elimination half-life ($t_{1/2\beta}$) is 32.9 ± 8.8 hours.[29, 47]

Table 2 Pharmacokinetic parameters of diazepam following intravenous administration.

Participants	n	Doses (mg/kg)	$t_{1/2\alpha}$ (min)	Vd (L/kg)	Clearance (L/hr/kg)	$t_{1/2\beta}$ (hr)	Ref.
Healthy adults	4	10 mg	1.9-13.3				34
Healthy adults	10	0.1		0.89±0.18	0.0384±0.015	32.9±8.8	48
Healthy adults	11	0.1		1.11 (0.70-1.78)	0.0234 (0.017-0.046)	36 (20.4-66.7)	49

$t_{1/2\alpha}$ = distribution half-life, **$t_{1/2\beta}$**= elimination half-life, **Vd** = Volume of distribution

Rectal Route

The rectal route is a well-tolerated and convenient route of administration. The rectal instillation of diazepam as a solution can provide reliable and rapid absorption. A C_{max} of 337 ng/mL was reached within 20 minutes in children (n = 5, age 11 to 15 years) after rectal administration of a solution of diazepam 0.12 to 0.26 mg/kg.[50] Effective anticonvulsant plasma concentrations (200 to 400 μg/L, chosen on somewhat arbitrary basis) were reached in infants (n = 10, age 10 to 24 months)

within 4 ± 1 minutes following rectal administration of solution of diazepam 0.7 mg/kg.[21]

Similar results were reported by others in infants and children,[51-54] in adult patient,[55] and in adult with epilepsy[56,57] and showed that diazepam as a solution was rapidly absorbed. However, the T_{max} was slightly longer in adults than in children.

The bioavailability of diazepam given by rectal instillation was found to be variable, and has been reported to be 50±17%[55], 80±13%[56] and complete. In contrast, diazepam suppositories led to erratic and low plasma concentrations and are not suitable for emergency treatment.[54, 58, 59]

Table 3 Pharmacokinetic parameters of diazepam following rectal administration of a solution.

Participants	n	Age (yr)	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (min)	Ref.
Neurological patients	6	24-60	10mg	201±73	16±3	55
Epileptic adults	6	19-39	10mg	309±68	20	56
Epileptic adults	6	18-39	10mg	305±67	36±20	57
Epileptic children	5	11-15	0.12-0.26	337	20	50
Epileptic children	10	10-24 mo	0.7	1449±177	10±2	21
Epileptic children	11	2wk-11	0.5	845±154	6	51
Ill Children	3	3-12	1	470-696	10[a]	52
Children[b]	22	1-9	0.4-0.5	206.7±105	11±1[a]	53
Epileptic children	9	1.4-15.2	0.4±0.14	404±253	10[a]	54

[a] Concentration measured at a fixed time after rectal administration

[b] Undergoing minor surgical procedures.

C_{max} = peak plasma drug concentration, **mo** = month; **n** = number of participants; **T_{max}** = time to reach peak concentration after drug administration; **wk** = week

Influence of Maturation

From combined data, Morselli et al.[60] showed that the $t_{1/2\beta}$ of diazepam was longer in premature neonates (54 hours) and shorter in children (18 hours) than in adults.

Efficacy of Diazepam in Treatment of Acute Seizures or Status Epilepticus.

In 1989 Treiman[61] reviewed the clinical efficacy of diazepam in management of one or several varieties of status epilepticus using the data from 47 studies involving 1455 patients. All studies supported a marked efficacy, measures as the percentage of cases with lasting seizure control: 39 to 100% for diazepam. However, all studies, except for that of Leppick et al.[35] were nonrandomized uncontrolled trials. The only blinded study was undertaken by Leppick et al.[35] and found similar results among adult patients: seizures were controlled in 76% of the episodes treated with intravenous diazepam 10 mg.

Most recent studies on the efficacy of benzodiazepines have been of nonblinded design; only 3 out of 18 studies were controlled.[62-64] The efficacy varied with route of administration: 28.6 to 100% for intrarectal diazepam and 54 to 100% of intravenous diazepam.[40,62-68]

A recent randomized double-blind study[68] demonstrated the statistically significant superiority of diazepam rectal gel (n = 46) for patients with acute repetitive seizures. The doses were 0.5 mg/kg for children 2 to 5 years old, 0.3 mg/kg for children between 6 and 11 years and 0.2 mg/kg for patients 12 years and older. A second dose was given in children and adults, and the third dose was given 8 hours after the second. Seizure frequency was reduced significantly in the active treatment group compared with the placebo group, the patients treated with diazepam rectal gel had a significantly longer mean time to their next seizure compared with those in the placebo group.

Route of Administration of Diazepam and Other Benzodiazepines in Treatment of Acute Seizure

None of these drugs is absorbed fast enough by oral route to be used in an emergency setting.

Intravenous administration as a bolus, short-term infusion or continuous infusion is preferable to other routes of administration, as the rapid delivery of the drug allows for an optimal concentration to be reached as soon as possible. However establishing intravenous access in a child with seizures is often difficult; this explains why other routes have been evaluated.

Rectal administration of benzodiazepines, specifically rectal instillation of a parenteral solution of diazepam, has been widely used. Although rectal absorption is rapid, the onset of action is delayed and bioavailability is highly variable, making this route not always totally reliable. However the feasibility, effectiveness and tolerability of rectal diazepam make it useful in the prehospital management of pediatric status epilepticus, as well as for home use for cluster and prolonged seizures. After rectal administration, the maximum concentrations of clonazepam, lorazepam and midazolam are obtained with some delayed, precluding their use by this route.[69]

Midazolam is the only benzodiazepine stable in aqueous solution and suitable for intramuscular injection. A delayed onset of action might be expected, as shown by Jawad et al.[70], but this was not confirmed by Chamberlain et al.[67] Intramuscular midazolam may be useful in patients when attempts to introduce an intravenous line are unsuccessful. It appeared to be well tolerated and rapidly effective for treatment of acute seizures.

Intranasal administration of midazolam was recently used in management of acute childhood seizures. The onset of action was rapid, as the meantime to eliminate spike activity on EEG was 111.5±95.3 seconds. This is the only available study using intranasal route for administration of an anticonvulsant drug and it should, therefore, be further evaluated. Furthermore, the intranasal parenteral solution of midazolam is not always locally well tolerated,[71] possibly because of the acid pH of the solution.

In 1998, Scott RC et al.[72] had studied the pharmacokinetics and EEG pharmacodynamics of buccal absorption of midazolam in 10 healthy volunteers, open-label and double-blind phases. Subjects held 10 mg midazolam in 2 mL peppermint-flavored fluid or peppermint-flavored placebo in their mouth for 5 minutes and then spat it out. Cardiorespiratory and EEG monitoring was performed in

all subjects. The result had shown the rapid increase of midazolam blood concentration for the first 20-30 min. However, changes in the 8 to 30 Hz frequencies identified by spectral analysis of the EEG showed changes in less or 5 to 10 min in test but not in control subjects. It is more rapid than were expected from the venous absorption data. After that, the randomized trial compared efficacy of buccal midazolam and rectal diazepam for treatment of prolonged seizures in childhood adolescence was performed in 1999.[73] The result showed the response to buccal midazolam occurred in 30(75%) in 40 episodes and response to rectal diazepam occurred in 23 (59%) of 39 episodes ($p = 0.16$). Analysis of the nine pairs of first treatment with each drug showed no significant difference in efficacy between treatments ($p = 0.38$) or in time from administration to end of seizure ($p = 0.28$).

Although no difference in efficacy between buccal midazolam and rectal diazepam was noted in this study, further work is needed before this mode of therapy is totally adopted. The patients ranged in age from 5 to 18 years, but single dose of each treatment was used for every patient. Whether differences in response rate would have emerged if the dose was based on weight (as is usual for children) is not clear.

A word of caution is also necessary about inappropriate extrapolation of the results. The patients in this study had chronic epilepsy and did not have status epilepticus at the time they were given the drug.[74] Whether either buccal midazolam or rectal diazepam would have been as effective in previously seizure-naive patients presenting with status epilepticus is not known.

Adverse Effects Related to Drug Concentration.[75,76]

Mild to severe respiratory depression was reported in 12% of patients receiving intravenous diazepam.[35] The rate of bolus injection is a critical factor, and an injection rate below 2 to 5 mg/min will seldom result in serious respiratory depression.[38] In a retrospective study in children with status epilepticus, the need for mechanical ventilation following intravenous treatment with diazepam were 36% of patients (5 of 14), 2.2±1.8 years old, with a dose of 0.38±0.21 mg/kg[65]

Gustafson and associates[77] from the Minnesota Epilepsy Group, St. Paul, noted that among 78 children given 532 doses of 0.5 mg/kg (maximum single dose, 20 mg) of rectal diazepam, no clinically significant respiratory depression was seen.

Blood concentrations and clinical effects of diazepam have been studied in some detail. Daily 10 mg doses of diazepam usually produce serum levels of less than 1 μg/mL. Therapeutic levels range between 0.5 and 2.0 μg/mL but vary widely. When measured for academic or forensic purposes, diazepam levels commonly reach 2 to 5 μg/mL in overdose case but may reach 10 to 15 μg/mL. Diazepam levels of 5 to 20 μg/mL are generally regarded as toxic, but many patients with serum concentrations in this range manifest only minimal clinical effects.

An apparently rare lethal intoxication by diazepam in combination with other drugs has been described in an addict. Massive oral dose may lead to cardiac arrest, hypotension, apnea, and coma. Patients intoxicated with plasma concentrations of 20,000 ng/mL of diazepam and 5,000 ng/mL of *N*-desmethyldiazepam and concentrations of oxazepam and *N*-methyloxazepam above 1,000 ng/mL have survived. Rapid clinical recovery from diazepam overdose does not result from rapid elimination, as both *N*-desmethyldiazepam and *N*-methyloxazepam have a long elimination half-life, but is more likely the result of tolerance to the depressant effect of the drug. Patient were fully alert at plasma concentrations of 1,800 to 7,000 ng/ml of diazepam and 600 to 5,000 ng/mL of *N*-desmethyldiazepam 1 to 2 days after a massive overdose. In a diazepam-induced coma, bullous skin lesions and exocrine sweat gland necrosis may occur. Apart from standard intensive care treatment, exchange transfusion and physostigmine have been employed in diazepam intoxication.

Dosage

In adult and children the recommended initial intravenous dose of diazepam for treatment of acute seizures is 0.2 to 0.3 mg/kg injected slowly until the seizure is stopped or serious hypotension or respiratory depression precludes any further administration. The maximal rate of intravenous diazepam should be 2 mg/min or 4 mg/min, and the maximal first dose should be 10 mg in adult patients.

As first aid, diazepam solution may be administered rectally at the recommended dose of 0.5 mg/kg.[69] In some literature noted that the rectal dose ranges from 0.2 to 2 mg/kg; the maximum initial dose should be 10 mg[78] , but some noted that when parenteral solutions of diazepam are administered rectally for management of status epilepticus, the usual dosage in adult and children is 0.5 mg/kg (not exceed 20mg).[11,29,81,82] However, another anticonvulsant, most often phenytoin, should be administered either simultaneously or immediately afterward.

CHAPTER III

PATIENTS AND METHOD

The Study was conducted from October 2000 to January 2002 at Queen Sirikit National Institute of Child Health, Bangkok, Thailand.

Patients

This study was designed as an opened-label, randomized, 2-way crossover trial to compare the pharmacokinetics of diazepam between buccal and rectal administration in epileptic children. The study protocol was reviewed and approved by the review board at Queen Sirikit National Institute of Child Health and the ethics committee of the Ministry of Public Health. The subjects of this study were selected from a group of epileptic patients at the Queen Sirikit National Institute of Child Health. Written informed consent had to be given by the parents or his legal guardian. The epileptic patients were recruited for this study based on the following criteria:

Inclusion Criteria

The patients who had all of these characteristics were enrolled in this study.

1. Epileptic patients associated with seizures, which could not be controlled who admitted in Hospital and volunteers.
2. An age of 3-15 years.
3. The parents or his legal guardian consented to enroll in this study

Exclusion Criteria

The patients who had either one of these characteristics were excluded from this study.

1. The patients had concomitant therapy with benzodiazepines.

2. The patients had received benzodiazepines and stopped for less than 3 weeks prior to starting of the study.
3. The patients had significantly changed or unstable vital sign.
4. The patients had significantly impaired liver and/or renal function.
5. The patients who had known allergy to diazepam.
6. The patients were diagnosed from physicians to be inappropriate to enroll in this study.

Number of Subjects

As Moolenaar et al.[58] have reported, peak plasma concentration (C_{max}) of diazepam after rectal administration was 369 ± 58 ng/mL. In order to compare the absorption of diazepam between the two routes, we made the assumption that if the C_{max} obtained after buccal route is 20% higher or lower than those obtain from rectal route, it will be concluded that there is significant difference in absorption ability between the two routes. Sample size was calculated from the equation as follow:

$$n = \frac{(z_{\alpha} + z_{\beta})^2 \times s_p^2}{D^2}$$

Where n = number of subjects

S_p^2 = pooled varience

D = difference

= 20% of 369 ng/mL = 73.8 ng/mL

$Z_{\alpha\ 0.05}$, two-tailed = 1.96

$Z_{\beta\ 0.1}$, one-tailed = 1.28

$$n = \frac{(1.96 + 1.28)^2 \times 58^2}{73.8^2}$$

$$n = 6.48 \cong 7$$

The number of subjects of at least 7 patients in each group was needed in this study. However, several others studies[21,50,51] had reported larger variations among subjects, resulted in larger standard deviation which means that a larger number of subjects is

required, this study decided to at least double the number of subjects to cover for these fluctuation, resulted in a number of twenty subjects to be included in the study.

Method

Study Design and Sample Collection

Twenty epileptic children who met the aforementioned criteria were participated in this study. They were divided in two groups, ten of them received diazepam via buccal route and ten of them received diazepam via rectal route for treatment of acute seizure. The dosages used were 0.5 mg/kg but the total maximum dose was kept to be not over 10 mg. After washed-out period, at least one month later, the route of administration would be altered for each group.

Series of plasma samples were collected from the patients after diazepam administration via either route. Two milliliters of blood samples were collected at time 0 minute (before taking diazepam), 5, 10, 15, 30, 60, 240, and 480 minutes after administering diazepam. The plasma portions were separated and kept at –70°C until analyzed. Clinical data of heart rate, pulse rate, blood pressure and consciousness were monitored and recorded at the time that blood samples were drawn. The time for seizures to stop after diazepam administration were defined for the patients who had acute seizure. If the seizure continued for longer than 5 minutes after drug administration, the treatment was deemed to have been ineffective and standard treatment was administered, according to the attending physician.

Method of Administration

Diazepam intravenous solution, 10 mg/2 mL produced by Government Pharmaceutical Organization (GPO), were used in either routes of administration.

For administration via buccal route, diazepam solution was drawn into syringe, the patient was hold to lie down on his side or in the prone position with his

head to one side, lips were parted and the diazepam solution was squirted around the buccal mucosa. The face was hold still for approximately 5 minutes after administration, then diazepam was sucked off the oral cavity to prevent choking.

Rectal diazepam was administered through of NG tube No.10 connected to syringe. First, bend upper leg forward to expose rectum, separate buttocks to expose rectum and gently insert NG tube tip into rectum for 4-5 cm. Pushing plunger and remove NG tube from rectum. Holding buttocks together still for 5 minutes before to prevent leakage.

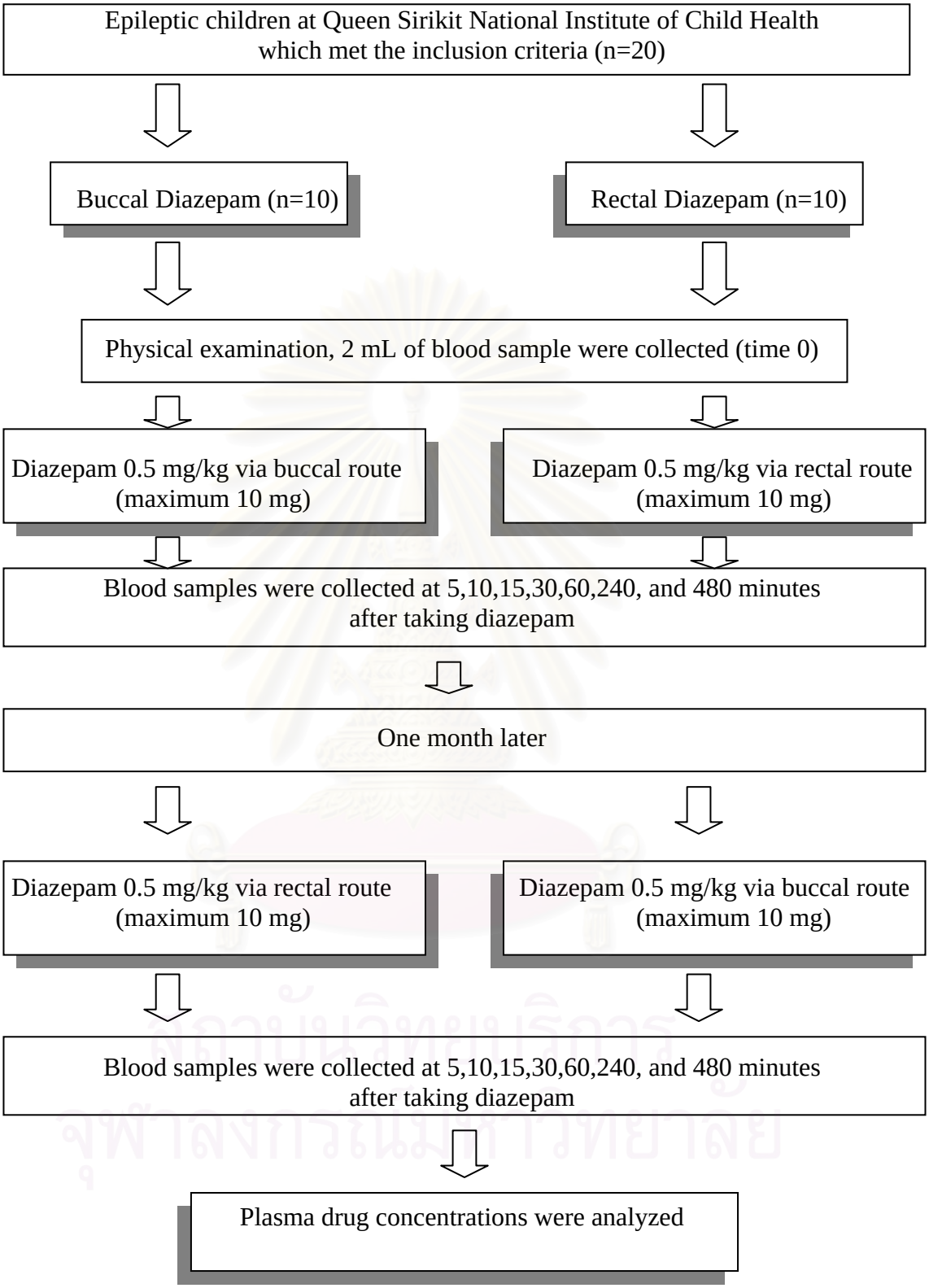

Figure 1 Flow chart of the study

Drug Assay

Concentrations of diazepam in plasma samples were quantified using the High Performance Liquid Chromatography (HPLC). Various concentrations of standard diazepam, i.e., 25, 50, 100, 250, 500, 1000 ng/mL were added to blank plasma from normal subject in order to generate the standard curve.

Extraction

The plasma samples extraction method was modified from the methods of Raisys VA et al.[79] and Brodie LR et al.[80]

One milliliter of plasma sample was added into a screw-capped tube filled with 1.0 mL of saturated KCL solution, 50μL of Clonazepam (10 μg/mL) was added to use as an internal standard (IS), vortex for 5 seconds to thoroughly mix the aqueous phase. The drugs were then extracted into 3 mL of toluene by shaking for about 30 minutes on a reciprocating shaker and centrifuge at 4000 rpm for 15 minutes. The organic layer was then transferred to a clean tube and evaporated to dryness by evaporator (Savant^{R}; Speed Vac). The residue was reconstituted with 200 μL of MeOH, sonicated for 30 seconds and then 20 μL aliquot was injected onto the HPLC column.

High Performance Liquid Chromatography

The Chromatograph consisted of an HPLC pump (Thermoseparate^{R}; Spectra system P1500) with UV detector (Thermoseparate^{R}; Spectra system UV 2000) set at 254 nm operated at maximum sensitivity of 0.05 a.u.f.s. A Waters reversed-phase column μBondapack C_{18} (10 μm particle size) 30 cm X 3.9 mm I.D. was maintained at room temperature. A Waters guard column C_{18} μBondapack was connected to the inlet end of the column. The mobile phase was methanol (MeOH)-water (65:35, v/v) at flow-rate of 1 mL/min. In these conditions, Internal standard, clonazepam and diazepam were eluted with retention times of 5.4 and 10.2 minutes, respectively.

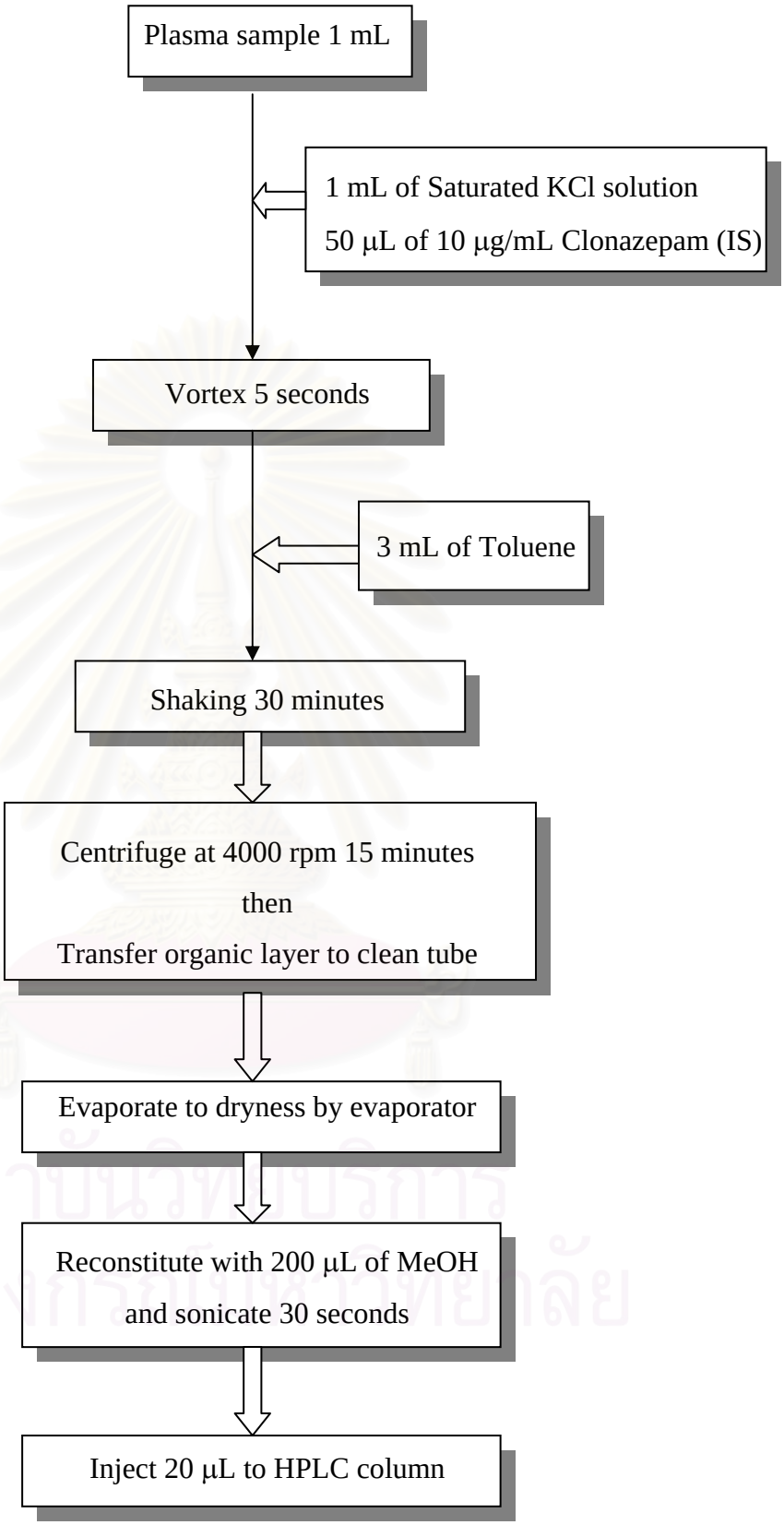

Figure 2 Method of plasma samples extraction

Pharmacokinetic Parameters

The data composed of 8 concentrations at various times after diazepam was administered, 0,5,10,15,30,60,240 and 480 minutes. The pharmacokinetic parameters of diazepam via buccal and rectal administration were derived, i.e., the maximum plasma concentration (C_{max}), time to reach C_{max} (T_{max}), the area under the concentration-time curve ($AUC_{0\text{-}8hr}$), absorption rate constant (K_a), elimination rate constant (K_e) and the half-life ($T_{1/2}$), using RSTRIP version 2.0 program which is the program for compartmental modeling and kinetic analysis for PC.

Statistical Analysis

The data of general characteristics and information obtained from the subjects including laboratory data were recorded and analyzed using descriptive statistics.

In order to compare the absorption of diazepam between the two routes, we made the assumption that if the C_{max} obtained after buccal administration is at least 20% higher or lower than those obtain from rectal route, it will be concluded that there is significant difference in absorption ability between the route. In addition, comparisons of area under the curve (AUC), time to reach the maximum concentration (T_{max}) and absorption rate constant (K_a) between routes of administration were performed by repeated measures ANOVA.

CHAPTER IV

RESULTS AND DISCUSSION

1. Study Population

During October 2001 and January 2002, 20 epileptic children from Queen Sirikit National Institute of Child Health were enrolled in the study. The consent forms were signed by their parents to participate in the study. They were divided into two groups, ten of them received diazepam via buccal route and ten of them received diazepam via rectal route to treat acute seizure in the dosage of 0.5 mg/kg but not over 10 mg. Blood samples were collected periodically and diazepam concentrations were analyzed. The pharmacokinetic parameters were then determined. After at least one month, washed-out period, the route of administration would be altered for each group. Blood samples were again collected, analyzed for their concentrations and the pharmacokinetic parameters were again determined.

Demographic Data

Of the 27 epileptic children recruited, twenty epileptic children completed study. Twelve of them were female (60%), the age range was 3-13 years and the mean age was 9.45 ± 3.56 years (mean ± S.D.). The mean weight was 30.08 ± 17.26 kg (mean ± S.D.) and the range was 12-79 kg. The dose received varied between 0.13-0.50 mg/kg with 7 of them received 0.5 mg/kg. Epileptic children in this study were diagnosed to be Lennox-Gastuat syndrome, Tuberous sclerosis, myoclonic seizure, generalized seizures, complex partial seizures, post encephalitis and some of them were unclassifiable. Laboratory data of the all patients, i.e., serum albumin, alanine aminotransferase (ALT), blood urea nitrogen (BUN) and serum creatinine indicated their liver and renal functions were within the normal ranges. Demographic data and types of seizures were shown in Table 4 and 5.

Table 4 Demographic data

Data (n=20)	Mean ± S.D.	Maximum	Minimum
Age (year)	9.45 ± 3.56	13.83	3.58
Weight (kg)	30.08 ± 17.26	79	12
Dose (mg/kg)	0.37 ± 0.13	0.50	0.13

Table 5 Summarizes the descriptive characteristics of the epileptic children who were enrolled in the study

No.	Sex	Age(yrs)	Wt (kg)	Dose(mg /kg)	Epilepsy Diagnosis	Type of Seizure	AEDs
1.	F	10.92	30.5	0.33	Post encephalitis	CPS, tonic, partial → 2^o generalized	PHT, PB
2.	F	13.00	25	0.4	Lennox-Gastaut syndrome	Partial → 2^o generalized	PHT, VPA
3.	M	5.00	15	0.46	Unclassifiable	Partial → 2^o generalized	PHT, PB
4.	M	11.00	30	0.33	Unclassifiable	Tonic	PHT, VPA
5.	M	11.00	32	0.31	Complex partial seizure	CPS	PHT
6.	M	11.00	18	0.5	Lennox-Gastaut syndrome	CPS, atonic, partial → 2^o generalized	VPA, PB
7.	M	6.50	27.5	0.36	Unclassifiable	Tonic, myoclonic	VPA
8.	F	3.58	12	0.5	Lennox-Gastaut syndrome	Partial → 2^o generalized, tonic	PHT, VPA, PB
9.	M	13.75	57	0.18	Complex partial seizures	CPS	CBZ
10.	F	13.83	79	0.13	Tuberous sclerosis	CPS, partial → 2^o generalized	PHT, VPA
11.	F	3.75	12	0.5	Generalized seizure	GTC	PHT, PB
12.	F	13.66	29	0.34	Unclassifiable	Tonic, atonic	VPA, PB
13.	M	6.08	16	0.5	Myoclonic seizure	Myoclonic	VPA
14.	F	8.00	16	0.5	Unclassifiable	Partial → 2^o generalized	VPA
15.	F	8.83	30	0.33	Unclassifiable	CPS, partial → 2^o generalized	CBZ, PB
16.	M	13.00	39	0.26	Unclassifiable	Partial → 2^o generalized, tonic	CBZ
17.	F	12.00	49	0.2	Unclassifiable	Partial → 2^o generalized	PHB
18.	F	5.92	20	0.5	Generalized tonic	Tonic	PHT, PHB
19.	F	5.75	16	0.5	Lennox-Gastaut syndrome	Partial → 2^o generalized, tonic, Infantile spasm, myoclonic	PHT, VPA, TPM
20.	F	12.42	49	0.2	Complex partial seizure	CPS	CBZ, PB

AEDs = Antiepileptic Drugs, Wt = Weight, M= Male, F= Female,
CPS = complex partial seizure, Partial → 2^o generalized = Partial seizure evolving to secondary generalized seizure,
PHT = Phenytoin, VPA = Sodium Valproate,
PB = Phenobarbital, CBZ = Carbamazepine, TPM = Topiramate

2. The Standard Curve of Diazepam in Plasma

After several steps of extraction according to the modified method of Raisys et al.[79] and Brodie et al.[80] as mentioned in detail in chapter III, the samples were injected into HPLC column. The retention time of diazepam and clonazepam were approximate 10.54 and 5.72 minutes respectively. The chromatograms were shown in figure 3, 4, and 5.

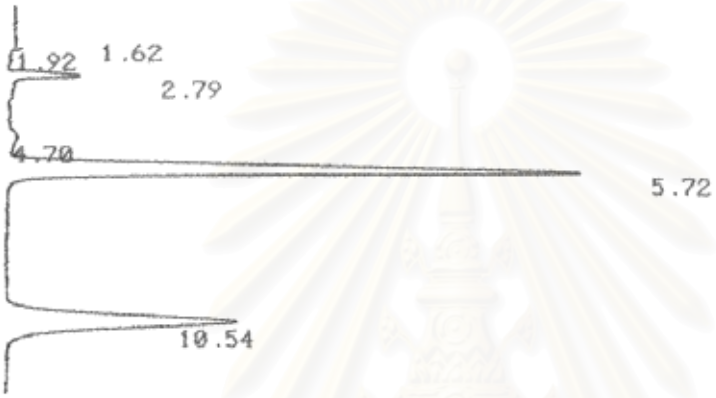

Figure 3 Chromatogram of diazepam 250 ng/mL and internal standard from standard solution.

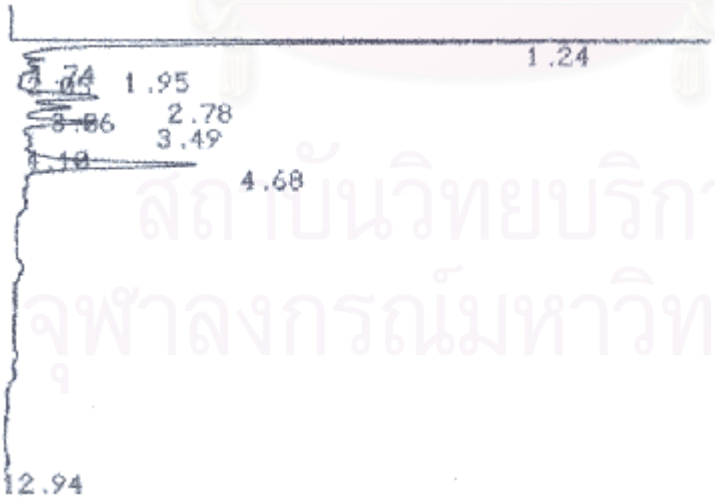

Figure 4 Chromatogram of blank plasma

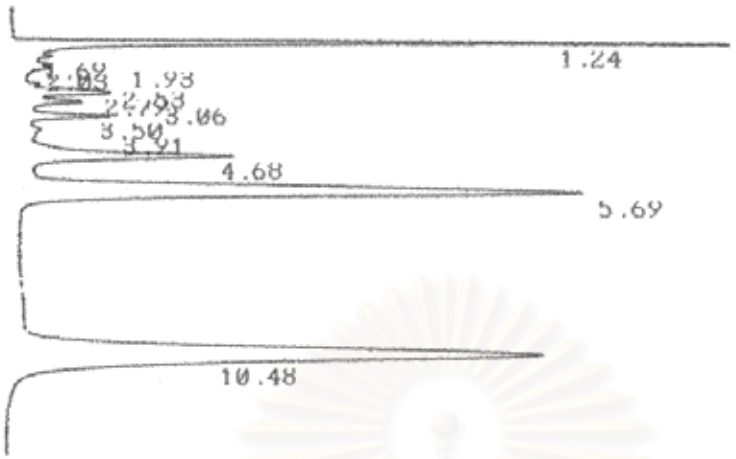

Figure 5 Chromatogram of diazepam 500 ng/mL and internal standard in plasma sample.

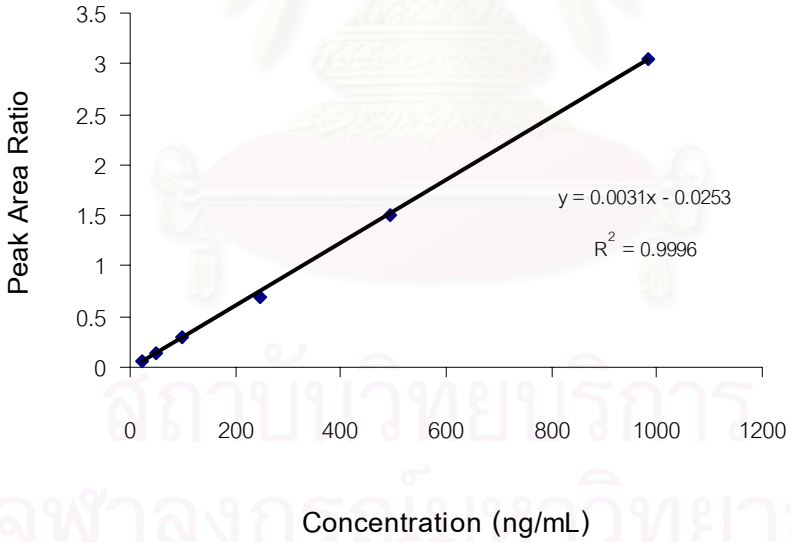

Figure 6 The standard curve of different concentrations of diazepam versus peak area ratio

The chromatographic conditions proved to be acceptable since a good separation of diazepam and clonazepam was obtained as shown in figure 3 and 5. Increased concentrations of diazepam resulted in a linearly increase in peak area ratio of diazepam to clonazepam (r^2 = 0.9996). The standard curve of diazepam is thus plotted as a line of best fit (Figure 6). The concentration of diazepam in each plasma sample was calculated from this standard curve by using the following equation:

Peak area ratio = 0.0031(Concentration)- 0.0253

As displayed in table 6, the average percentage of recovery of the analysis procedure was 99.63 %. The precision and accuracy of the a fore mentioned procedure was satisfactory since the coefficient of variation (%CV) were found to be 2.64% for within-run and 4.79% for between-run.

Table 6 Coefficient of variation (%CV) and % recovery of plasma diazepam concentration analysis

DiazepamConcentration (ng/mL)	**Within-Run %CV**	**Between-Run % CV**	**% Recovery**
25	5.10	8.38	97.59
500	1.02	2.05	99.85
1000	1.81	3.96	101.46
Mean	**2.64**	**4.79**	**99.63**

3. **Pharmacokinetics of Diazepam after Buccal and Rectal Routes of Administration**

The blood samples were collected from twenty epileptic children at various times after received diazepam by buccal or rectal routes of administration, i.e., at 0, 5,10, 15, 30, 60, 240, and 480 minutes after diazepam was administered. The dosage given was 0.5 mg/kg but the maximum total dose was kept at 10 mg.

The mean diazepam concentration (mean ± S.D.) at various times after buccal and rectal administrations were presented in table 7 and figure 7. From the data obtained it was found that the maximum concentration (C_{max}) of diazepam after buccal administration was 220.47 ± 140.47 ng/mL and was obtained at the time of 15 minutes while for rectal route, the C_{max} was found to be 268.41 ± 190.81 ng/mL at the time of 10 minutes.

Table 7 Mean concentrations of diazepam in plasma at various times after buccal and rectal administration with dosage of 0.5 mg/kg (maximum dose 10 mg)

Time (min)	**Mean Diazepam Concentrations Mean ± S.D. (ng/mL)**	
	Buccal Route	**Rectal Route**
0	0	0
5	134.33 ± 136.48	178.82 ± 139.33
10	206.93 ± 142.69	268.41 ±190.80
15	220.47 ± 140.47	263.47 ± 153.87
30	196.64 ± 115.22	191.86 ± 110.28
60	147.84 ± 85.51	158.00 ± 84.05
240	100.99 ± 52.22	103.53 ± 50.63
480	64.69 ± 45.55	70.19 ± 44.39

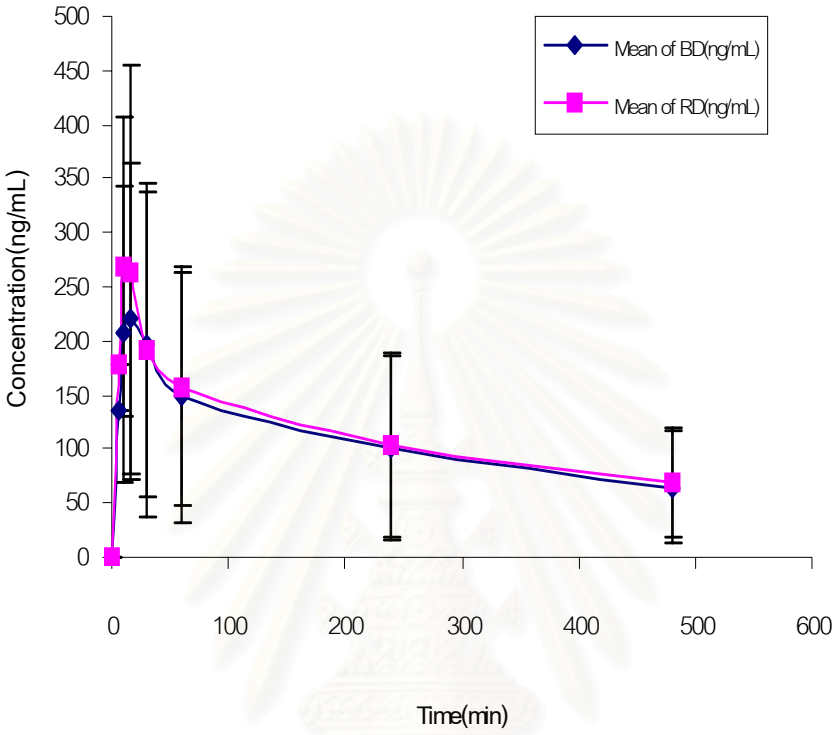

Figure 7 Mean diazepam concentrations in plasma versus time curves after buccal (BD) and rectal (RD) administrations of diazepam in the dosage of 0.5 mg/kg (maximum dose 10 mg)

Pharmacokinetics parameters of diazepam after buccal and rectal administrations were derived from 2 methods, i.e., non-compartmental analysis (method A) and compartmental analysis derived from RSTRIP program (method B).

Method A : Non-Compartmental Analysis

Diazepam pharmacokinetic parameters after buccal and rectal administrations were calculated from the data of individual patients (as showed in table A1 andA2 of appendix A). The pharmacokinetic parameters consisted of maximum concentration (C_{max}), time to reach C_{max} (T_{max}), and area under the curve from the time of 0 minutes to 8 hours ($AUC_{0\text{-}8hr}$) which calculated from trapezoidal integration. The data were shown in table 8.

The maximum concentrations (C_{max}) after rectal and buccal administrations of each subject were compared by repeated measures ANOVA. The results revealed the mean C_{max} after buccal and rectal diazepam administrations to be 264.07 ± 149.53 and 314.84 ± 180.33 ng/mL, respectively, and showed that there were no statistically significant differences between the two routes of administration (P = 0.184). Data of individual patient showed that there were nine patients whose C_{max} was higher after buccal administration than rectal administration while the other eleven patients were vice versa. There were also high variations of C_{max} after administration by both routes. However, 90% confident interval (90% CI) of ln C_{max} ratio showed that C_{max} after buccal diazepam administration was between 65 to 104% of rectal diazepam administration (90%CI = 0.6573 to 1.0452).

The data also revealed that 90% confident interval of ln $AUC_{0\text{-}8\ hr}$ after buccal administration was between 28% lower and 15% higher than rectal administration (90% CI = 0.7150 to 1.1540) however, no statistical significance were found in the difference of $AUC_{0\text{-}8hr}$ between the two routes according to the repeated measures ANOVA (P = 0.678).

The means of time to reach maximum concentration after administration (T_{max}) were faster after rectal administration than after buccal administration and were shown to be statistically significant different (11.5 ± 5.64 and 15.75 ± 7.83 minutes; P = 0.031).

Table 8 Diazepam pharmacokinetic parameters of individual patients after buccal and rectal administrations derived from non-compartmental analysis.

Number	Dose	**Buccal Administration**			**Rectal Administration**		
	(mg/kg)	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0\text{-}8hr}$ ($ngml^{-1}min$)	C_{max} (ng/mL)	T_{max} (min)	$AUC_{0\text{-}8hr}$ ($ngml^{-1}min$)
1	0.33	258.01	30	47689	243.73	30	62666
2	0.4	370.36	15	50503	227.82	15	61283
3	0.46	605.94	15	122947	535.84	15	66422
4	0.33	377.63	30	63736	466.35	15	46979
5	0.31	313.81	15	78316	810.31	10	84749
6	0.5	242.84	10	55295	429.74	10	108331
7	0.36	415.98	10	69195	232.43	10	43019
8	0.5	520.69	10	78070	392.45	15	31677
9	0.18	374.67	15	89305	442.42	10	83958
10	0.13	78.24	30	12894	215.46	5	32578
11	0.5	250.83	10	52675	449.57	10	123851
12	0.34	103.35	15	23594	256.49	10	49372
13	0.5	185.84	5	19200	365.06	5	59850
14	0.5	286.48	15	82808	92.03	15	31647
15	0.33	164.98	10	36436	117.99	10	27304
16	0.26	54.80	15	16108	137.58	10	41305
17	0.2	106.47	15	37421	155.56	15	36295
18	0.5	278.02	30	54243	265.86	5	53993
19	0.5	200.99	10	45654	387.75	10	61776
20	0.2	91.37	10	20481	72.31	5	11803
Mean		264.07	15.75	52828.5	314.84	11.5	55942.9
S.D.		149.53	7.83	28462.4	180.33	5.64	27712.53
Max		605.94	30	122947	810.31	30	123851
Min		54.80	5	12894	72.31	5	11803

Table 9 Comparisons of pharmacokinetic parameters of diazepam between buccal and rectal administration from non-compartmental analysis.

Parameters	Route	Mean ± S.D.	90%CI of ln ratio	Significance
C_{max} (ng/mL)	Buccal	264.07 ±149.53	0.6573 to 1.0452	P = 0.184
	Rectal	314.84 ±180.33		
T_{max} (min)	Buccal	15.75 ± 7.83	-	P = 0.031
	Rectal	11.5 ± 5.64		
AUC_{0-8hr} ($ngml^{-1}min$)	Buccal	52828.5 ± 28462.4	0.7150 to 1.1540	P =0.678
	Rectal	55942.9 ± 27712.53		

Method B : Compartmental Analysis by RSTRIP Program

Pharmacokinetic parameters of diazepam after buccal and rectal administrations were calculated from the data of individual patients (as showed in table A1 and A2 of appendix A) by RSTRIP version 2.0 program. The results were shown in table 10 and 11. Maximum concentration (C_{max}), time to reach C_{max} (T_{max}) and area under the curve (AUC) of this method were reported from stripping and fitting data by RSTRIP program.

Table 10 Diazepam pharmacokinetic parameters of individual patients after buccal administration derived from RSTRIP program (compartmental analysis).

Number	C_{max} (ng/mL)	T_{max} (min)	K_a (hr^{-1})	K_e (hr^{-1})	$T_{1/2}$ (hr)	$AUC_{0\text{-}8hr}$ ($ngml^{-1}min$)	$AUC_{0\text{-}\infty}$ ($ngml^{-1}min$)
BD01	210.81	26.44	8.46	0.2400	2.8618	49372	57990
BD02	313.98	18.64	4.332	0.2556	2.7080	15911	15911
BD03	512.94	7.95	40.065	0.2036	3.4047	124679	155301
BD04	277.98	37.71	5.04942	0.2736	2.5330	63496	72093
BD05	303.82	36.14	5.86854	0.2092	3.3132	79340	98563
BD06	183.23	17.49	17.1672	0.1218	5.6928	57967	93538
BD07	327.47	21.77	10.098	0.3104	2.2332	64590	70692
BD08	317.97	0.00	0.169482	0.1614	4.2943	83671	112868
BD09	279.65	25.85	10.9356	0.1071	6.4718	93662	163997
BD10	67.19	22.33	8.9826	0.3578	1.9375	12115	12881
BD11	191.36	14.99	19.7736	0.1488	4.6595	55543	80094
BD12	73.46	12.38	27.0564	0.1039	6.6740	24396	43359
BD13	83.39	0.00	0.245166	0.2335	2.9687	18144	21472
BD14	276.26	26.33	9.9294	0.1348	5.1417	85472	130449
BD15	181.21	18.34	3.18414	0.3033	2.2857	9501.4	95014
BD16	59.77	36.84	5.12514	0.2536	2.7332	14249	16535
BD17	106.57	3.40	131.634	0.0766	9.0458	38383	83812
BD18	247.44	48.14	3.17226	0.3438	2.0162	52498	56576
BD19	159.79	3.66	110.304	0.1336	5.1870	47468	72332
BD20	66.75	20.16	14.7108	0.1136	6.1003	21739	36610
Mean	212.05	19.93	21.81314	0.2043	4.1131	50609.82	74504.35
SD	118.16	13.29	35.40388	0.0864	1.9445	31913.19	44156.99
Max	512.94	48.14	131.634	0.3578	9.0458	124679	163997
Min	59.77	0.00	0.169482	0.0766	1.9375	9501.4	12881

The results revealed that C_{max} after buccal administration was 212.05 ± 118.6 ng/mL and after rectal administration was 250.85 ± 132.26 ng/mL with no statistically

significant difference (P = 0.203) whereas 90% CI of ln C_{max} ratio showed that C_{max} after buccal administration was between less than 35% and 8% higher than those obtained after rectal administration. (90%CI = 0.6556 to 1.0835).

Table 11 Diazepam pharmacokinetic parameters of individual patients after rectal administration derived from RSTRIP program (compartmental analysis).

Number	C_{max} (ng/mL)	T_{max} (min)	$\mathbf{K_a}$ (hr^{-1})	K_e (hr^{-1})	$T_{1/2}$ (hr)	$AUC_{0\text{-}8hr}$ ($ngml^{-1}min$)	$AUC_{0\text{-}\infty}$ ($ngml^{-1}min$)
RD01	238.15	50.04	3.47766	0.2422	2.8623	60845	72029
RD02	174.74	34.20	8.1408	0.0894	7.7572	62281	123359
RD03	452.09	15.79	5.244	0.2753	2.5175	20471	20471
RD04	334.31	14.70	5.01876	0.3498	1.9815	13214	13214
RD05	427.41	0.00	0.397578	0.2499	2.7732	86772	243399
RD06	367.32	12.36	26.0694	0.1245	5.5653	114199	181552
RD07	170.52	14.67	4.53216	0.4062	1.7067	43326	54998
RD08	274.17	14.76	4.8183	0.3655	1.8962	10723	10723
RD09	351.12	14.85	18.5478	0.2003	3.4603	88004	110506
RD10	133.70	2.97	127.416	0.2201	3.1488	30498	36839
RD11	425.10	20.93	13.0554	0.1472	4.7085	125538	182350
RD12	177.63	7.02	49.4754	0.1638	4.2307	48340	66227
RD13	329.89	1.42	278.61	0.3917	1.7695	48774	50998
RD14	92.24	50.66	3.47574	0.0755	9.1757	33521	67795
RD15	98.92	15.04	18.7614	0.1798	3.8557	26256	34533
RD16	110.76	3.30	137.556	0.1082	9.7880	40719	94209
RD17	129.54	15.40	18.7632	0.1584	4.3767	36594	51114
RD18	253.80	3.17	116.166	0.2544	2.7250	52730	60677
RD19	436.01	18.94	6.5484	0.1273	5.4435	30504	30505
RD20	39.65	2.38	186.714	0.1157	5.9915	12465	20657
Mean	250.85	15.63	51.6394	0.2123	4.2867	49288.7	76307.75
SD	132.26	14.46	76.90733	0.1026	2.3880	32425.54	63249.07
Max	452.09	50.66	278.61	0.4062	9.7880	125538	243399
Min	39.65	0.00	0.397578	0.0755	1.7067	10723	10723

Area under the curve after administration to 8 hours (AUC_{0-8hr}) were 50609.82 ± 31913.19 and 49288.7 ± 32425.54 $ngml^{-1}min$ after buccal and rectal administrations, respectively. There were no significant difference between AUC_{0-8hr} (P = 0.893) and $AUC_{0-\infty}$ (P = 0.915) of the two routes. However, 90% CI of ln AUC ratios showed that AUC_{0-8hr} after buccal administration was between 68 to 147 % of rectal administration (90% CI = 0.6816 to 1.4770) while $AUC_{0-\infty}$ after buccal routes was between 68 to 170% of rectal route (90% CI = 0.6816 to 1.7073).

When the absorption rate constant (K_a) were compared by repeated measures ANOVA, it was found that there were no statistically significant difference between buccal route and rectal route (P = 0.153). In case of T_{max}, the result showed that buccal administration reached the maximum concentration slower than rectal administration 19.93 ± 13.29 and 15.63 ± 14.26 minutes, however, this difference was not statistically significant (P = 0.35).

The data revealed half-life of diazepam to be 4.11 ± 1.94 and 4.29 ± 2.39 hours after buccal and rectal administration respectively. These results were consistent with a previous study by Tungnararutchakit K.[83] which reported the half-life of diazepam in 1 to 3 year-old Thai children after oral administration to be 4.16 ± 3.59 hours. However, literature review indicated the elimination half-life ($T_{1/2\beta}$) of diazepam in children 2 to 12 years to be about 15-21 hours [60] which was different from the results in this study. The present study calculated the parameter from only 8 points of sampling time with the last sample collected at 8 hours only after drug administration, therefore, the data was fitted by the pharmacokinetics computer program (RSTRIP) to be a one compartment model. If the blood samples were collected further for a longer period of time, the second compartment might show up and could result in a longer reported half-life. Clearance and volume of distribution could not be calculated from this study, since these parameters related to the amount of drug entered general circulation, while in this study the drug was sucked out of the buccal cavity after 5 minutes to prevent choking, so the amount of drug that actually reached blood circulation could not be predicted.

Table12 Comparisons of pharmacokinetic parameters of diazepam from compartmental analysis between buccal and rectal administration

Parameters	Route	Mean ± S.D.	90%CI of ln ratio	Significance
C_{max} (ng/mL)	Buccal	212.05 ± 118.16	0.6556 to 1.0835	P = 0.203
	Rectal	250.85 ± 132.26		
T_{max} (min)	Buccal	19.93 ± 13.29	-	P = 0.350
	Rectal	15.63 ± 14.46		
K_a (hr^{-1})	Buccal	21.81 ± 35.40	-	P = 0.153
	Rectal	51.64 ± 76.91		
AUC_{0-8hr} ($ngml^{-1}min$)	Buccal	50609.82 ± 31913.19	0.6806 to 1.4770	P = 0.893
	Rectal	49288.7 ± 32425.54		
$AUC_{0-\infty}$ ($ngml^{-1}min$)	Buccal	74504.35 ± 44156.99	0.6816 to 1.7073	P = 0.915
	Rectal	76307.75 ± 63249.07		

4. Correlation Between Age and Dose on C_{max} and AUC

No statistical correlation was found between dose (mg/kg) to C_{max} and T_{max} of diazepam after either route of administration. However, correlation between dose (mg/kg) to C_{max} after buccal administration was nearly significant (P=0.061), if the number of subjects was increased and/or the dose range was wider (in this study = 0.13 to 0.5 mg/kg), significant correlation might be found.

There were statistically significant inversely correlation between age and C_{max} after buccal administration (r = - 0.4952, P = 0.026) and the correlation between age and $AUC_{0\text{-}8hr}$ after buccal administration was nearly significant (r = -0.3992, P=0.081). However, statistically significant correlation between dose and age on C_{max} and $AUC_{0\text{-}8hr}$ after rectal administration could not be found in this study.

Although statistical analysis found the correlation between age and C_{max} after buccal administration was significant, this might cause by the relationship of dose and age, i.e., the higher age often got the lower dose in mg/kg since the maximum dose was locked at 10 mg, therefore, the older child with a heavier weight usually end up with lower dose in mg/kg. Although the literature review showed that the half-life of diazepam was shorter in children than adult [60], there were no statistically significant correlation between age and elimination rate constant (K_e) after buccal administration among the children participated in this study (r = -0.0682, P = 0.775). Therefore, the correlation between age and C_{max} could possibly be related to the correlation between dose and C_{max}.

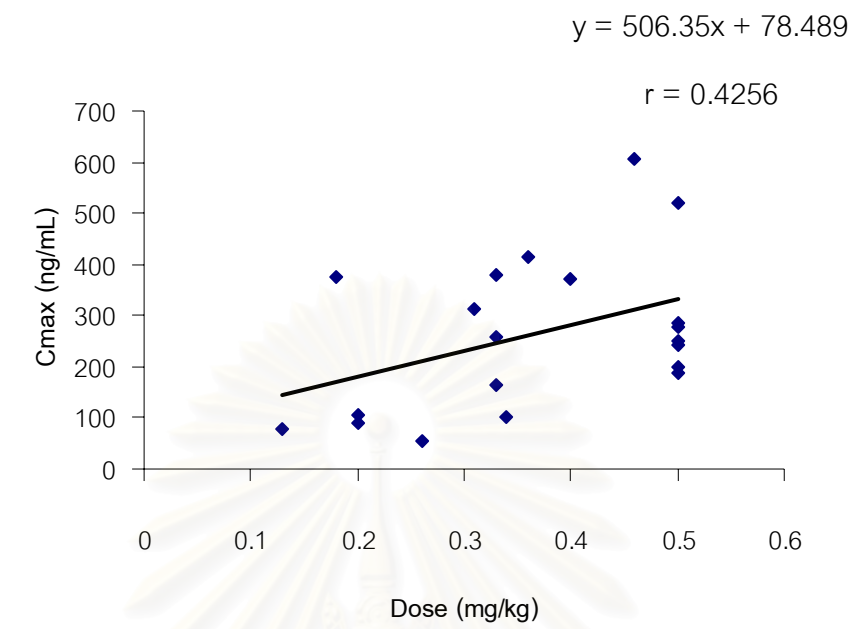

Figure 8 Correlation between dose (mg/kg) and C_{max} after buccal administration

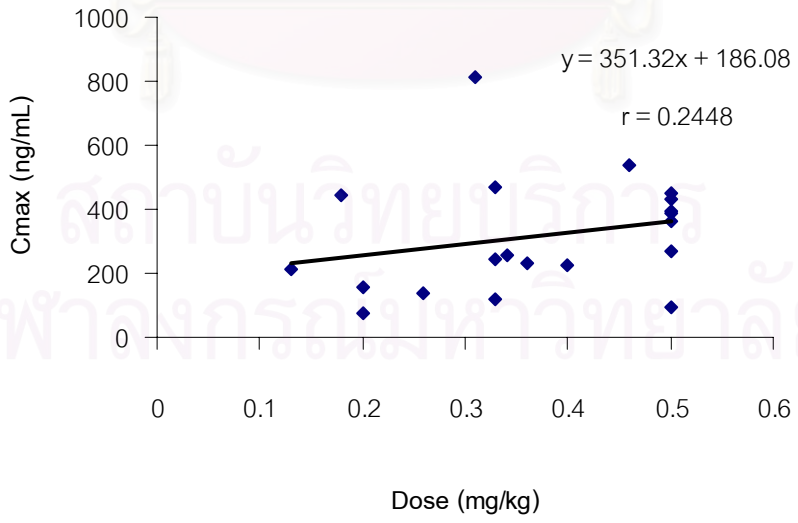

Figure 9 Correlation between dose (mg/kg) and C_{max} after rectal administration

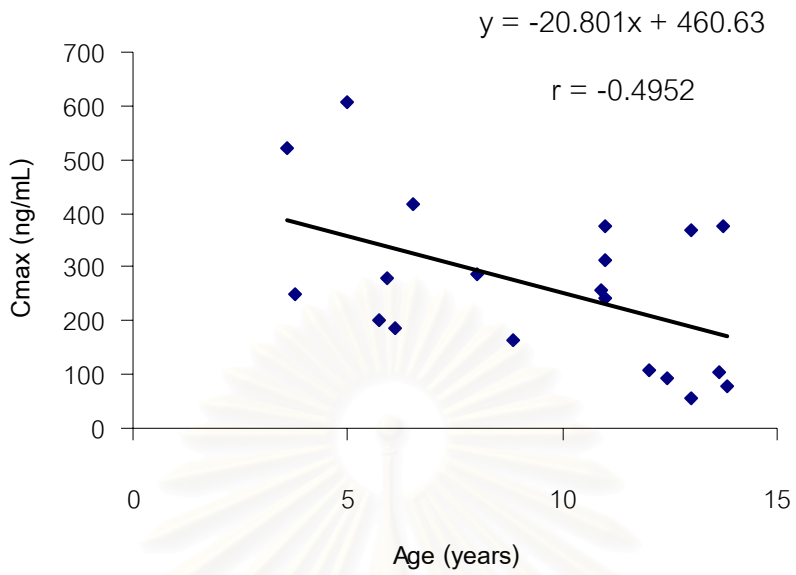

Figure 10 Correlation between age and C_{max} after buccal administration

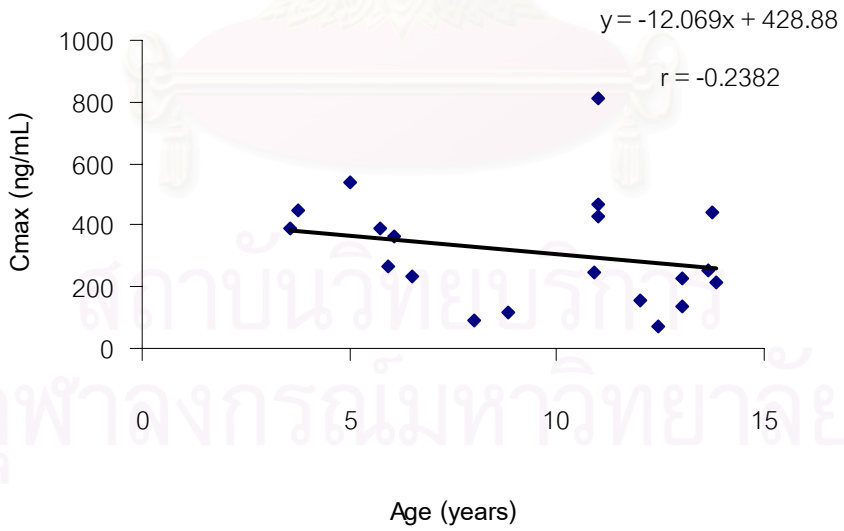

Figure 11 Correlation between age and C_{max} after rectal administration

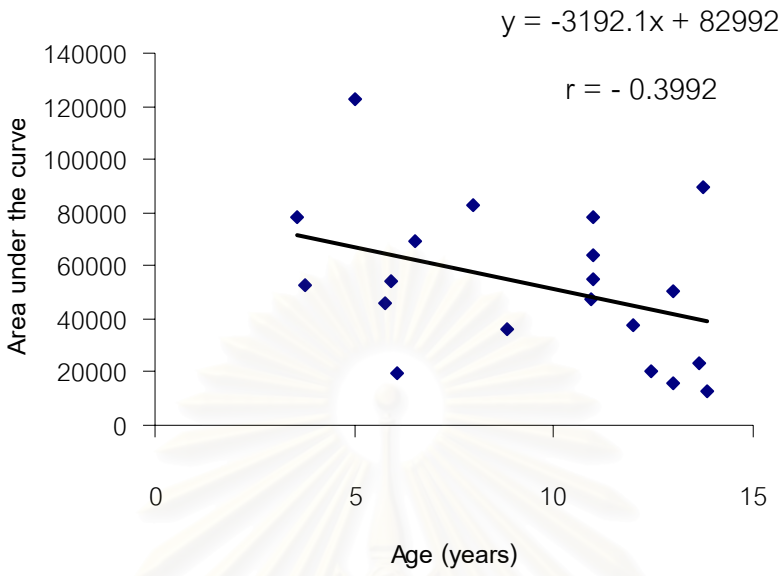

Figure 12 Correlation between age and $AUC_{0\text{-}8hr}$ after buccal administration

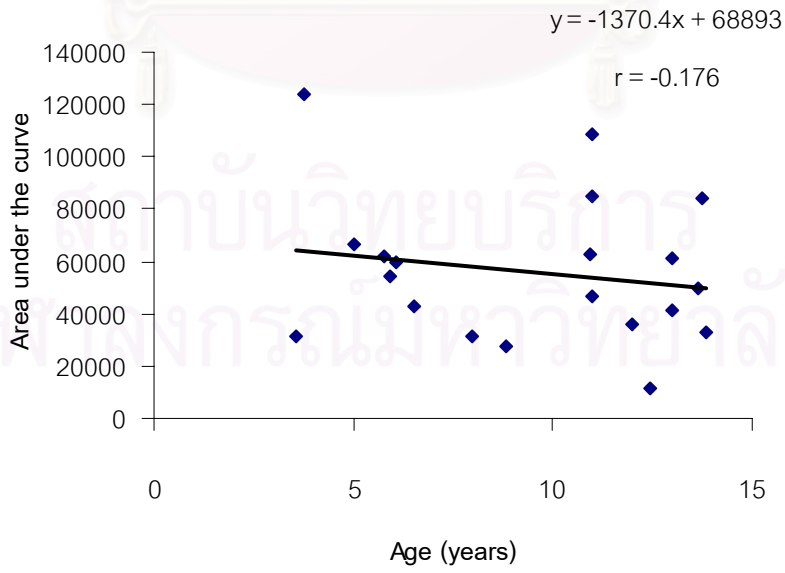

Figure 13 Correlation between age and $AUC_{0\text{-}8hr}$ after rectal administration

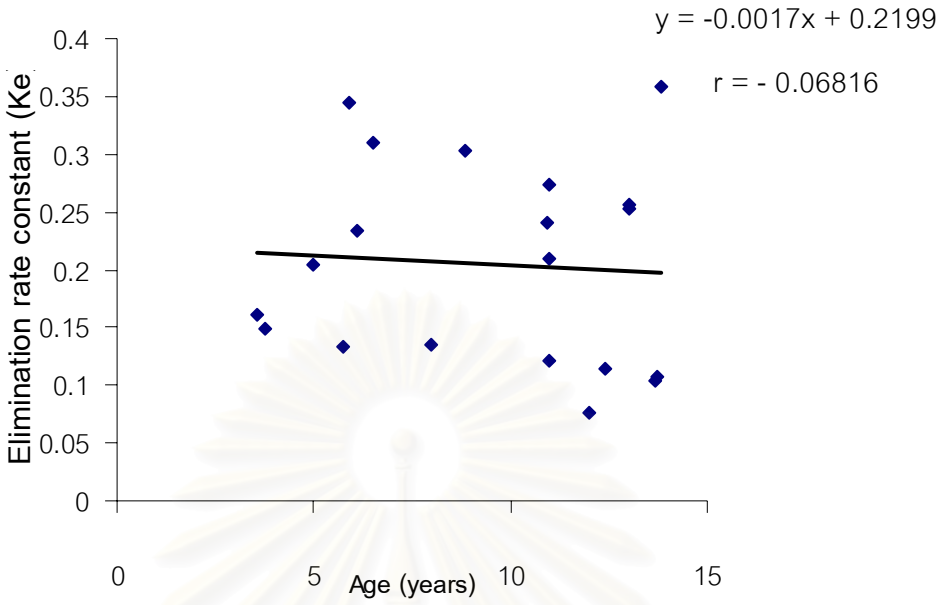

Figure 14 Correlation between age and elimination rate constant (K_e)

5. Clinical Effects of Diazepam after Buccal and Rectal Administration

Clinical Efficacy

The efficacy of diazepam intrarectal was reported to be 28.6 to 100% [69] while another study indicated that seizure frequency was reduced significantly and the mean time to their next seizure was significantly longer after rectal gel diazepam compared to the placebo group.[68]

In this study, most patients had chronic epilepsy but did not show the status epilepticus during the time they joined in this study and were given the drug. There were only nine children who were having acute seizures during the period of study, however, majority of each episode occurred and ceased in a few minutes, so the real clinical efficacy could not be evaluated (table 9). All of these nine patients, however, showed no re-occurrence of any clinical seizures beyond 12 hours after the administration of diazepam via either route.

Table 13 Time to the end of seizure after diazepam were administered via buccal and rectal routes

No.	Dose (mg/kg)	Buccal Route		Rectal Route	
		Arrival time of nurse	Time since drug administration to the end of seizure	Arrival time of nurse	Time since drug administration to the end of seizure
2	0.4	5 min	4 min	45 sec	4 min
3	0.46	50 sec	1 min	40 sec	1 min
4	0.33	2 min	15 sec	1 min	1 min
6	0.5	50 sec	15 sec	1 min	1 min
7	0.36	12 sec	15 sec	50 sec	30 sec
8	0.5	20sec	5sec	30 sec	20 sec
10	0.13	1 min	1 min	10 sec	5 sec
11	0.5	10 sec	30 sec	45 sec	30 sec
12	0.34	50 sec	1.5 min	40 sec	3 min

In treatment of acute seizures, little data are available to define the effective drug plasma concentration of diazepam, the minimum plasma concentration required to suppress seizures is thought to range between 200 to 600 ng/mL in most emergency setting. And 200 ng/mL is the concentration considered necessary to control status epilepticus in human. [38-40, 75]

The number of patients whose maximum concentrations (C_{max}) after buccal and rectal diazepam could reach the target concentrations, 500 ng/mL to be able to terminate seizure and 200 ng/mL to be able to control seizure, were displayed in table 8. The results revealed that there were only 2 out of 20 patients (10%) in each group whose C_{max} level reached the level of 500 ng/mL, however, most patients showed their C_{max} level to be above 200 ng/mL [13 (65%) and 15 (75%) patients after buccal and rectal administrations, respectively].

Considering the time to reach target concentration, the number of patients whose plasma level reached 500 ng/mL within 5 minutes after administration were only two after buccal administration and only one after rectal administration. Two patients after buccal administration reached the target of 200 ng/mL, while 5 patients after rectal administration reached this level of target concentrations within 5 minutes. Within 10 minutes, the number of patients reached the target of 500 ng/ml were not changed, but for 200 ng/mL target concentration, there were 10 patients (50%) after buccal administration and 12 patients (60%) after rectal administration respectively reached this target. Plasma diazepam levels were lower than 200 ng/mL in majority of the patients (95%) at 4 hours and in all patients at 8 hours after drug administration via either routes (table15).

Table 14 The number of patients whose plasma diazepam levels reached target concentrations

Time (min)	Concentration (ng/mL)	Number of Patients	
		Buccal Administration (n = 20)	Rectal Administration (n = 20)
5	**> 500**	2	1
	> 200	2	5
10	**> 500**	2	1
	> 200	10	12

Table 15 The number of patients whose plasma diazepam level was lower than 200 ng/mL at various times after drug administration

Time (minutes)	Number of Patients	
	Buccal Administration (n = 20)	Rectal Administration (n = 20)
30	9	10
60	14	14
240	19	19
480	20	20

As presented above, C_{max} of diazepam after buccal administration were not different from those obtained after rectal administration. Even though the dose was fixed at 0.5 mg/kg in the beginning, due to caution about the safety of patients since the absorption via buccal route was not known, the physician wanted to keep the maximum total dose to be not over 10 mg per one time, therefore, the dosage administration were varied from 0.13 to 0.5 mg/kg. However, data showed that the two patients who reached target concentration of 500 ng/mL within 5 minutes after buccal administration were received the dose of 0.46 and 0.5 mg/kg which mean that the higher the dose administration the higher would be the concentration. Assumed that concentrations were linearly related to the doses, if the doses in mg/kg were adjusted in all patients to be 0.5 mg/kg, the predicted diazepam concentrations at various times would be as presented in table 16 and figure 15

Table 16 Predicted mean concentrations of diazepam in plasma at various times after adjusted the dosage of all patients to 0.5 mg/kg

Time (min)	Mean Diazepam Concentrations Mean ± S.D. (ng/mL)	
	Buccal Administration	**Rectal Administration**
0	0	0
5	194.88 ± 153.53	282.17 ± 256.08
10	297.18 ± 164.52	392.09 ± 325.64
15	344.22 ± 231.38	372.24 ± 228.58
30	299.30 ± 168.09	277.37 ± 186.88
60	225.41 ± 136.64	232.29 ± 133.14
240	159.43 ± 99.24	154.24 ± 83.84
480	103.18 ± 92.64	105.14 ± 78.88

From the adjusted data, it was found that C_{max} of most subjects after either buccal or rectal administrations could reach the levels which believed to be able to terminate and/or control the seizure. C_{max} obtained from the mean concentrations were equal to 344.22 ± 231.38 at the time of 15 minutes and 392.09 ± 325.64 ng/mL at the time of 10 minutes for buccal route and rectal route respectively. The means of individual C_{max} were 382.82 ± 176.91 ng/mL after buccal administration and 475.65 ± 321.95 ng/mL after rectal administration. Repeated measures ANOVA presented that these C_{max} obtained from both routes were not statistically significant difference ($P = 0.155$).

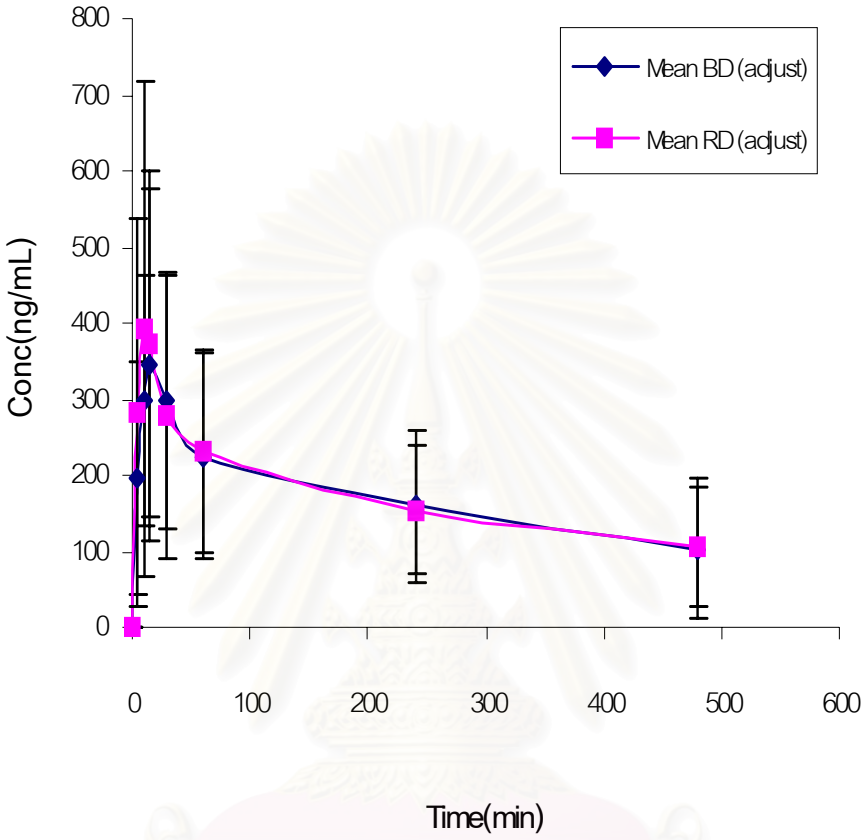

Figure 15 Predicted mean diazepam concentrations versus time curves after buccal (BD) and rectal (RD) administrations with the dosage of all patients adjusted to 0.5 mg/kg

Table 17 Predicted C_{max} and T_{max} of diazepam in plasma after buccal and rectal administrations after adjusted the dosage to 0.5 mg/kg

No.	Buccal Administration		Rectal Administration	
(n = 20)	C_{max} (ng/mL)	T_{max} (min)	C_{max} (ng/mL)	T_{max} (min)
1.	390.92	30	369.29	30
2.	462.95	15	284.78	15
3.	658.62	15	582.43	15
4.	572.17	30	706.58	15
5.	506.15	15	1306.96	10
6.	242.84	10	429.74	10
7.	577.74	10	322.82	10
8.	520.69	10	392.45	15
9.	694.85	15	1228.94	10
10.	300.92	30	828.69	5
11.	254.84	10	449.57	10
12.	607.93	15	377.19	10
13.	185.84	5	365.06	5
14.	286.48	15	92.03	15
15.	249.97	10	178.77	10
16.	105.38	15	264.57	10
17.	330.74	15	388.91	15
18.	278.02	30	265.86	5
19.	200.99	10	497.51	10
20.	228.42	10	180.78	5
Mean ± S.D.	**382.82 ± 176.91**	**15.75 ±7.83**	**475.65 ± 321.95**	**11.5 ± 5.64**
Range	**105.38 – 694.85**	**5 - 30**	**92.03 – 1306.96**	**5 – 30**

Table 18 Predicted number of patients whose plasma diazepam levels would reach the target concentrations after adjusted the dosage of all patients to 0.5 mg/kg

Time (min)	Concentration (ng/mL)	Predicted Number of Patients	
		Buccal Administration (n = 20)	Rectal Administration (n = 20)
5	**> 500**	2	3
	> 200	6	11
10	**> 500**	4	3
	> 200	16	15

From all of the data above, the absorption ability of diazepam via buccal route did not significantly different from the rectal route. Although the time to reach maximum concentration (T_{max}) via buccal route was a little bit more delayed as compared to the rectal route, high variation were found from both rectal and buccal diazepam administrations. C_{max} obtained after either route of administration were slightly lower than the target levels and were highly variable which might be due in part to the variation of dosage received by each patient since the maximum total dose was kept to be not over 10 mg per time. The number of patients reached the target concentration of 200 ng/mL and 500 ng/mL were not different between the two routes.

In this study, even though the patients had chronic epilepsy, they did not show the status epilepticus at the time they were given the drug. Their clinical efficacy could not be determined truly. Further studies in higher dosage at the time of seizure should be performed to observe the true effects on clinical outcome.

Adverse Effects

Majority of the patients complained about the bitter taste of diazepam when used via buccal administration. During the period of study, heart rate, blood pressure and respiratory rate were measured at the time that the blood samples were collected, no cardiovascular and respiratory depression were recorded in any subjects.

6. Feasibility of Buccal Diazepam to be an Alternative to Rectal Diazepam in Treatment of Acute Seizure

All data above showed that absorption ability of diazepam via buccal route seemed to be equal to rectal route. However, there were high variability among the patients in both routes, from this study we found the standard deviation of C_{max} after buccal administration to be 149.53 ng/mL which was quite wide, this implied that if the number of subjects were increased, some difference might be able to determine.

The dosage formulation used to administer via both buccal and rectal routes was the parenteral solution dosage form, most patients complained about the bitter taste of the formulation. However, this problem could be solved through a pharmaceutical research to develop a more pleasant flavor formulation. At the same time, due to the problem of aspiration (choking), the volume given to the patients while they are seizure should keep as minimum as possible. The concentration of the buccal formulation should therefore be increased from the present parenteral formulation.

Moreover, development of product preparation to a ready-to-administer preparation for buccal use might help solving the problem on the difficulty of drawing diazepam from ampules.

Same as rectal diazepam[20], buccal diazepam could be useful in the prehospital management of pediatric status epilepticus as well as for home use for cluster and prolonged seizures, however, in application, caregivers should be trained in administration technique to avoid aspiration. It seems to be feasible that buccal route of diazepam administration can be use as an alternative to rectal route especially after a better formulation has been developed.

CHAPTER V

CONCLUSION

1. Study Population

Twenty epileptic children were enrolled in the study. They were divided into two groups, ten of them received diazepam via buccal route and ten of them received diazepam via rectal route to treat acute seizure in the dosage of 0.5 mg/kg but not over 10 mg. After at least one month, the routes of administration were altered for each group.

Demographic Data

Twelve of the twenty patients were female, the age range was 3-13 years and the mean age was 9.45 ± 3.56 years (mean ± S.D.). The mean weight was 30.08 ± 17.26 kg (mean ± S.D.) and the range was 12-79 kg. The dose received varied between 0.13-0.50 mg/kg with 7 of them received 0.5 mg/kg. Epileptic children in this study were diagnosed to be Lennox-Gatuat Syndrome, Tuberous sclerosis, myoclonic seizure, generalized seizures, complex partial seizures, post encephalitis and some of them were unclassifiable. Laboratory data of all patients, i.e., serum albumin, ALT, BUN and serum creatinine were within the normal ranges.

2. The Standard Curve for Analysis of Diazepam in Plasma

The method for analysis of diazepam concentrations in plasma was developed by modified from those methods of Raisys et al. and Brodie et al. The standard curve was prepared by using standard diazepam solutions of various concentrations, i.e., 25, 50, 100, 250, 500, and 1000 ng/mL, with clonazepam as the internal standard. The retention time of diazepam and clonazepam were approximately 10.54 and 5.72

minutes, respectively. The correlation between concentration and peak area ratio of diazepam and clonazepam was y = 0.0031x - 0.0253 (r^2 = 0.9996).

3. Pharmacokinetics of Diazepam after Buccal and Rectal Administrations

C_{max} of diazepam from the mean diazepam concentrations at various times after buccal administration was 220 ± 140.47 ng/mL and appeared at 15 minutes while for rectal route, the C_{max} was 268.41 ± 190.81 ng/mL and was found at 10 minutes.

Method A: Non-compartmental analysis

Considered C_{max} obtained from individual patients, the mean C_{max} after buccal administration was 264.07 ± 149.53 ng/mL while the mean C_{max} was 314.84 ± 180.33 ng/mL after rectal administration. There were no statistical significant difference between the two routes of administration (P = 0.184). 90% confident interval of ln C_{max} ratio showed that C_{max} after buccal diazepam administration was between 65 to 104% of rectal diazepam administration (90%CI = 0.6573 to 1.0452).

No statistical significant difference of $AUC_{0\text{-}8hr}$ were found between the two routes (P =0.678), and ln $AUC_{0\text{-}8hr}$ of buccal administration was between less than 29% and 16% higher than rectal administration (90% CI = 0.7150 to 1.1540).

Mean T_{max} after diazepam was administered via buccal route was slower than that obtained after rectal administration significantly (15.75 ± 7.83 and 11.5 ± 5.64 minutes; P = 0.031).

Method B : Compartmental analysis by RSTRIP program

Mean C_{max} after buccal administration was 212.05 ± 118.6 ng/mL and was 250.85 ± 132.26 ng/mL after rectal administration with no statistically significant difference (P = 0.203) and 90% CI of ln C_{max} ratio showed that C_{max} after buccal route were 65 % to 108 % of those obtained after rectal administration.

T_{max} after buccal administration was slower than rectal administration (19.93 ± 13.29 minutes and 15.63 ± 14.26 minutes), but not statistically significant different.(P = 0.35)

$AUC_{0\text{-}8hr}$ were not significant different between the two routes (50609.82 ± 31913.19 and 49288.7 ± 32425.54 $ngml^{-1}min$ after buccal and rectal administrations respectively; P = 0.893)

Mean K_a was 21.81 ± 35.40 $hour^{-1}$ for buccal route and 51.64 ± 76.91 $hour^{-1}$ for rectal route with no statistical significant difference (P=0.153). Mean K_e was 0.2043 ± 0.0864 hr^{-1} and 0.2123 ± 0.1026 hr^{-1} after buccal and rectal administrations, respectively. Mean $T_{1/2}$ of diazepam was 4.11 ± 1.94 hours after buccal administration and was 4.29 ± 2.39 hours after rectal administration.

4. Correlation Between age and dose on C_{max} and AUC

No statistical significant correlation was found between dose (mg/kg) to C_{max} of diazepam after either route of administration, however, there was tendency that this correlation would be significant after buccal administration (P = 0.06139). There were significant inversely correlation between age and C_{max} after buccal administration but not significant between age and $AUC_{0\text{-}8hr}$ (r =-0.4952, P = 0.02642 and r = -0.3992, P = 0.08121 respectively). However, since there were no significant correlation between age and K_e, the effect of age to C_{max} could possibly be related to the inversely correlation between age and dose in mg/kg and could possibly be explained mostly by the correlation between dose in mg/kg and C_{max}.

5. Clinical Effects of Diazepam after Buccal and Rectal Administrations

Clinical Efficacy

There were only 2 out of 20 children after each route of administration whose C_{max} level reached 500 ng/mL. However, most patients, i.e., 13 patients after buccal administration and 15 patients after rectal administration, showed their C_{max} level to be above 200 ng/mL. Those two patients whose C_{max} reached the target concentration of 500 ng/mL after buccal administration, the target level were reached within 5 minutes while only one out of the two patients after rectal administration reached the target level within 5 minutes. Two patients after buccal administration reached the target concentration of 200 ng/mL within 5 minutes while 5 patients after rectal administration reached this same level within 5 minutes. Plasma diazepam levels were lower than 200 ng/mL in 95% of the patients at 4 hours and in all patients at 8 hours after drug administration via either route.

The dosages actually gave to the patients were varied from 0.13 to 0.5 mg/kg due to the lock of maximum dose to 10 mg per dose. If the dose in mg/kg were adjusted in all patients to be 0.5 mg/kg without locking the maximum, predicted mean of individual C_{max} would be 382.82 ± 176.91 ng/mL and 475 ± 321.95 ng/mL after buccal and rectal administrations respectively with no statistically significant difference ($P = 0.155$).

In this study, most patients had chronic epilepsy but did not show the status epilepticus during the time they joined in this study when the drug was given. Since majority of the episode of acute seizures occurred and ceased in a few minutes, the reliable clinical efficacy could not be evaluated.

Adverse Effects

Most patients complained about the bitter taste of the formulation. No cardiovascular and respiratory depression were recorded in any subjects.

6. Feasibility of Buccal Diazepam to be an Alternative to Rectal Diazepam in Treatment of Acute Seizure

Further study in higher dosage at the time of seizure should be performed to observe the true effects on clinical outcome. The problem about the bitter taste of the formulation could be solved through a pharmaceutical research. Due to the problem of aspiration, the volume gave to the patients while they have seizure should keep as minimum as possible, the concentration of buccal formulation should therefore be increased from the present parenteral formulation. A ready- to administer preparation for buccal use might overcome the difficulty of drawing diazepam from ampules as happened in the present study. It seemed to be quite feasible that buccal route of diazepam administration can be use as an alternative to rectal route of administration especially after a better formulation has been developed.

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APPENDICES

Appendix A

Table A1 Plasma diazepam concentrations at various times of individual patients after buccal administration.

Time(min)	BD01	BD02	BD03	BD04	BD05
0	0	0	0	0	0
5	50.08394	61.55685	500.8153	43.81364	49.11442
10	203.6562	308.9774	521.2544	145.2493	194.9839
15	218.2957	370.3578	605.9261	230.0385	313.8139
30	258.0077	216.179	451.3452	377.632	305.1969
60	113.9416	128.6609	318.5367	170.9053	269.9061
240	86.01999	107.5918	240.0007	142.7823	124.2836
480	70.03402	33.16254	148.1286	33.87635	105.4217
Time(min)	BD06	BD07	BD08	BD9	BD10
0	0	0	0	0	0
5	111.8898	69.59561	507.2423	126.9556	46.9402
10	242.8376	415.9763	520.6934	231.6532	45.72078
15	176.7111	343.5538	342.2706	374.6737	69.14832
30	169.0216	273.3677	245.704	250.1456	78.23943
60	145.8654	252.6144	181.1924	227.5066	41.65167
240	104.5443	111.738	152.0052	173.704	26.69227
480	89.00575	59.92499	108.6648	148.087	0
Time(min)	BD11	BD12	BD13	BD14	BD15
0	0	0	0	0	0
5	133.5262	69.80037	185.8446	172.9391	152.4472
10	250.8354	56.32912	90.11151	206.1399	164.9776
15	198.7168	103.3473	65.8928	286.4826	163.1338
30	179.7583	68.12455	60.68326	283.2619	128.0843
60	120.5884	52.36799	56.3548	259.5843	104.8735
240	107.6836	50.63621	47.03917	138.6074	60.40261
480	78.71448	35.53691	0	127.585	55.32753
Time(min)	BD16	BD17	BD18	BD19	BD20
0	0	0	0	0	0
5	28.45751	132.3098	44.64657	169.7725	28.81652
10	33.36338	101.0685	112.4575	200.9914	91.3695
15	54.79903	106.4688	139.9132	171.6716	74.10378
30	54.45317	88.77808	278.0232	107.0565	59.76184
60	54.73839	87.42502	222.4804	102.9832	44.5739
240	39.41973	79.178	84.58164	99.32584	43.55587
480	0	61.54287	39.00355	66.69478	33.05921

Table A2 Plasma diazepam concentrations at various times of individual patients after rectal administration.

Time(min)	RD01	RD02	RD03	RD04	RD05
0	0	0	0	0	0
5	55.25607	40.36772	158.3366	133.3368	609.8225
10	96.14352	122.1348	462.4006	283.9884	810.314
15	172.1947	227.8217	535.8357	466.3452	343.2939
30	243.7342	155.5196	267.4555	156.0997	309.7682
60	214.79	149.0912	159.3561	87.89979	235.345
240	133.4546	132.7555	122.9503	89.23927	149.1096
480	34.37378	92.99491	78.25364	72.888	93.51374
Time(min)	RD06	RD07	RD08	RD9	RD10
0	0	0	0	0	0
5	316.7107	109.618	106.0169	240.7231	215.4583
10	429.7376	232.4331	227.826	442.4178	105.9801
15	374.798	199.4314	392.4549	376.2098	105.0298
30	346.832	125.574	119.7483	328.1628	77.17923
60	285.3952	104.3167	76.98646	215.7679	109.5164
240	198.7521	93.05345	48.55973	143.2577	58.94014
480	177.8532	49.95362	42.56762	138.8033	35.89549
Time(min)	RD11	RD12	RD13	RD14	RD15
0	0	0	0	0	0
5	247.7954	172.9239	365.0564	0	71.22175
10	449.5714	256.4862	350.729	42.64413	117.9891
15	430.8512	149.0681	290.9762	92.03033	105.0659
30	399.8523	130.6258	209.6904	91.57009	65.3579
60	379.4186	122.2833	192.8221	88.09234	99.60174
240	224.3693	117.5789	99.25202	57.42921	48.02697
480	167.5186	43.8634	62.94753	59.53036	27.43861
Time(min)	RD16	RD17	RD18	RD19	RD20
0	0	0	0	0	0
5	110.2069	106.6997	265.8615	178.7246	72.3135
10	137.578	116.1535	263.2937	387.7459	32.69879
15	95.8911	155.5643	231.6747	497.507	27.37295
30	95.09205	117.4179	220.7874	349.0807	27.57877
60	95.49395	106.6907	182.1095	227.1712	27.80103
240	94.84994	68.40129	83.56553	83.63141	23.52018
480	58.51463	45.13564	61.6042	39.15943	21.04693

Table A3 Predicted plasma diazepam concentrations at various times of individual patients after buccal administration (adjusted dosage of all patients to 0.5 mg/kg).

Time(min)	BD01	BD02	BD03	BD04	BD05
0	0	0	0	0	0
5	75.88476	76.94606	544.3645	66.3843	79.21681
10	308.57	386.2218	566.5809	220.0746	314.4902
15	330.7511	462.9473	658.6154	348.5432	506.1515
30	390.9208	270.2237	490.5926	572.1696	492.2531
60	172.6387	160.8262	346.2355	258.9474	435.3324
240	130.3333	134.4897	260.8703	216.3368	200.4574
480	106.1122	41.45318	161.0094	51.32781	170.035
Time(min)	BD06	BD07	BD08	BD09	BD10
0	0	0	0	0	0
5	111.8898	96.66057	507.2423	352.6544	180.5392
10	242.8376	577.7449	520.6934	643.481	175.8491
15	176.7111	477.1581	342.2706	1040.76	265.9551
30	169.0216	379.6773	245.704	694.8489	300.9209
60	145.8654	350.8533	181.1924	631.9629	160.1987
240	104.5443	155.1917	152.0052	482.5112	102.6626
480	89.00575	83.22915	108.6648	411.3529	0
Time(min)	BD11	BD12	BD13	BD14	BD15
0	0	0	0	0	0
5	133.5262	410.5904	185.8446	172.9391	230.9806
10	250.8354	331.3478	90.11151	206.1399	249.9661
15	198.7168	607.9253	65.8928	286.4826	247.1725
30	179.7583	400.7327	60.68326	283.2619	194.0672
60	120.5884	308.047	56.3548	259.5843	158.8992
240	107.6836	297.8601	47.03917	138.6074	91.5191
480	78.71448	209.0407	0	127.585	83.82959
Time(min)	BD16	BD17	BD18	BD19	BD20
0	0	0	0	0	0
5	54.72599	330.7746	44.64657	169.7725	72.0413
10	64.16035	252.6713	112.4575	200.9914	228.4238
15	105.3827	266.1719	139.9132	171.6716	185.2595
30	104.7176	221.9452	278.0232	107.0565	149.4046
60	105.2661	218.5626	222.4804	102.9832	111.4348
240	75.80717	197.945	84.58164	99.32584	108.8897
480	0	153.8572	39.00355	66.69478	82.64804

Table A4 Predicted plasma diazepam concentrations at various times of individual patients after rectal administration (adjusted dosage of all patients to 0.5 mg/kg).

Time(min)	RD01	RD02	RD03	RD04	RD05
0	0	0	0	0	0
5	83.72131	50.45965	172.105	202.0254	983.5847
10	145.672	152.6685	502.6093	430.2855	1306.958
15	260.9011	284.7772	582.4301	706.5837	553.6998
30	369.2942	194.3995	290.7125	236.5147	499.6262
60	325.4394	186.3641	173.2132	133.1815	379.5887
240	202.204	165.9444	133.6417	135.211	240.4994
480	52.08149	116.2436	85.0583	110.4364	150.8286
Time(min)	RD06	RD07	RD08	RD09	RD10
0	0	0	0	0	0
5	316.7107	152.2473	106.0169	668.6753	828.686
10	429.7376	322.8238	227.826	1228.938	407.616
15	374.798	276.988	392.4549	1045.027	403.9606
30	346.832	174.4084	119.7483	911.5635	296.8432
60	285.3952	144.8844	76.98646	599.3554	421.2168
240	198.7521	129.2409	48.55973	397.9381	226.6929
480	177.8532	69.38003	42.56762	385.5646	138.0596
Time(min)	RD11	RD12	RD13	RD14	RD15
0	0	0	0	0	0
5	247.7954	254.2999	365.0564	0	107.9117
10	449.5714	377.1856	350.729	42.64413	178.7713
15	430.8512	219.2179	290.9762	92.03033	159.1908
30	399.8523	192.0967	209.6904	91.57009	99.02713
60	379.4186	179.8284	192.8221	88.09234	150.9117
240	224.3693	172.9102	99.25202	57.42921	72.76814
480	167.5186	64.505	62.94753	59.53036	41.57365
Time(min)	RD16	RD17	RD18	RD19	RD20
0	0	0	0	0	0
5	211.9363	266.7492	265.8615	178.7246	180.7838
10	264.573	290.3838	263.2937	387.7459	81.74698
15	184.406	388.9108	231.6747	497.507	68.43238
30	182.8693	293.5448	220.7874	349.0807	68.94694
60	183.6422	266.7268	182.1095	227.1712	69.50257
240	182.4037	171.0032	83.56553	83.63141	58.80046
480	112.5281	112.8391	61.6042	39.15943	52.61732

Appendix B

Repeated measures ANOVA

1. C_{max} after buccal and rectal diazepam administration (non-compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	264.1	149.5	33.44
Rectal route	20	314.8	180.3	40.32

Source of variation	SS	DF	Variance Est (MS)
Between subjects	7.844e+05	19	
Within Subjects	2.84e+05	20	
Treatment	2.578e+05	1	2.578e+04
Residual	2.582e+05	19	1.359e+04
Total	1.068e+06	40	

$$F = \frac{MStreat}{MSres} = \frac{2.578e+04}{1.359e+04} = 1.897$$

P = 0.184

2. T_{max} after buccal and rectal diazepam administration (non-compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	15.75	7.826	1.75
Rectal route	20	11.5	5.643	1.262

Source of variation	SS	DF	Variance Est (MS)
Between subjects	1137	19	
Within Subjects	812.5	20	
Treatment	180.6	1	180.6
Residual	631.9	19	33.26
Total	1949	40	

$$F = \frac{MStreat}{MSres} = \frac{180.6}{33.26} = 5.431$$

P = 0.031

3. $AUC_{0\text{-}8hr}$ after buccal and rectal diazepam administration (non-compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	5.283e+04	2.846e+04	6364
Rectal route	20	5.594e+04	2.771e+04	6197

Source of variation	SS	DF	Variance Est (MS)
Between subjects	1.959e+04	19	
Within Subjects	1.049e+10	20	
Treatment	9.699e+07	1	9.699e+07
Residual	1.039e+10	19	5.47e+08
Total	3.008e+10	40	

$$F = \frac{MStreat}{MSres} = \frac{9.699e+07}{5.47e+08} = 0.177$$

P = 0.678

4. C_{max} after buccal and rectal diazepam administration

(Method B : Compartmental analysis)

Group	N	Mean	S.D.	SEM
Buccal route	20	212.1	118.2	26.42
Rectal route	20	250.9	132.3	29.58

Source of variation	SS	DF	Variance Est (MS)
Between subjects	4.331	19	
Within Subjects	1.797e+05	20	
Treatment	1.505e+04	1	1.505e+04
Residual	1.646e+05	19	8664
Total	6.127e+05	40	

$$F = \frac{MStreat}{MSres} = \frac{1.505e+04}{8664} = 1.738$$

P = 0.203

5. T_{max} after buccal and rectal diazepam administration

(Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	19.93	13.29	2.972
Rectal route	20	15.63	14.46	3.233

Source of variation	SS	DF	Variance Est (MS)
Between subjects	3515	19	
Within Subjects	3998	20	
Treatment	184.7	1	184.7
Residual	3814	19	200.7
Total	7514	40	

$$F = \frac{MStreat}{MSres} = \frac{184.7}{200.7} = 0.920$$

P = 0.350

6. $AUC_{0\text{-}8hr}$ after buccal and rectal diazepam administration

(Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	5.061e+04	3.191e+04	7136
Rectal route	20	4.929e+04	3.243e+04	7251

Source of variation	SS	DF	Variance Est (MS)
Between subjects	2.16e+10	19	
Within Subjects	1.775e+10	20	
Treatment	1.745e+07	1	1.745e+07
Residual	1.773e+10	19	9.332e+08
Total	3.934e+10	40	

$$F = \frac{MStreat}{MSres} = \frac{1.745e+07}{9.332e+08} = 0.019$$

P = 0.893

7. $AUC_{0-\infty}$ after buccal and rectal diazepam administration

(Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	7.631e+04	6.325e+04	1.414e+04
Rectal route	20	7.45e+04	4.416e+04	9874

Source of variation	SS	DF	Variance Est (MS)
Between subjects	6.07e+10	19	
Within Subjects	5.239e+10	20	
Treatment	3.252e+07	1	3.252e+07
Residual	5.236e+10	19	2.756e+09
Total	1.131e+11	40	

$$F = \frac{MStreat}{MSres} = \frac{3.252e+07}{2.756e+09} = 0.012$$

P = 0.915

8. K_a after buccal and rectal diazepam administration

(Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	0.858	1.284	0.287
Rectal route	20	0.3507	0.5946	0.133

Source of variation	SS	DF	Variance Est (MS)
Between subjects	15.9	19	
Within Subjects	24.69	20	
Treatment	2.574	1	2.574
Residual	22.12	19	1.164
Total	40.6	40	

$$F = \frac{MStreat}{MSres} = \frac{2.574}{1.164} = 2.211$$

P = 0.153

90% Confident Interval

1. C_{max} after buccal and rectal diazepam administration (non-compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	264.1	149.5	33.44
Rectal route	20	314.8	180.3	40.32

Source of variation	SS	DF	Variance Est (MS)
Between subjects	7.844e+05	19	
Within Subjects	2.84e+05	20	
Treatment	2.578e+05	1	2.578e+04
Residual	2.582e+05	19	1.359e+04
Total	1.068e+06	40	

$$F = \frac{MStreat}{MSres} = \frac{2.578e+04}{1.359e+04} = 1.897$$

P = 0.184

$$90\%CI = \bar{\Delta} \pm t\sqrt{EMS\left(\frac{1}{N1}+\frac{1}{N2}\right)}$$

$$90\%CI = -50.7722 \pm 1.734\sqrt{1.359\times10^{4}\left(\frac{1}{20}+\frac{1}{20}\right)}$$

$$90\%CI = (-114.6954),(13.151)$$

$$90\%CI = \frac{314.8376+(-114.6954)}{314.8376}, \frac{314.8376+13.151}{314.8376}$$

$$90\%CI = 0.6357, 1.0418$$

Where EMS = Estimated Mean Square

= Residual Mean Square

$t_{0.1}$, df = 18 =1.734

2. ln C_{max} after buccal and rectal diazepam administration (non-compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	5.582	0.6285	0.1405
Rectal route	20	5.394	0.6615	0.1479

Source of variation	SS	DF	Variance Est (MS)
Between subjects	12.42	19	
Within Subjects	3.75	20	
Treatment	0.3524	1	0.3524
Residual	3.398	19	0.1788
Total	16.17	40	

$$F = \frac{MStreat}{MSres} = \frac{0.3524}{0.1788} = 1.970$$

P = 0.177

$$90\%CI = \bar{\Delta} \pm t\sqrt{EMS\left(\frac{1}{N1}+\frac{1}{N2}\right)}$$

$$90\%CI = (-0.1877) \pm 1.734\sqrt{0.1788\left(\frac{1}{20}+\frac{1}{20}\right)}$$

$$90\%CI = (-0.4196), (0.0442)$$

anti ln

$$90\%CI = 0.6573, 1.0452$$

; Where EMS = Estimated Mean Square

= Residual Mean Square

$t_{0.1}$, df = 18 = 1.734

3. ln $AUC_{0\text{-}8hr}$ after buccal and rectal diazepam administration (non-compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	10.7	0.67	0.1498
Rectal route	20	10.81	0.5366	0.12

Source of variation	SS	DF	Variance Est (MS)
Between subjects	10.38	19	
Within Subjects	3.738	20	
Treatment	0.1212	1	0.1212
Residual	3.617	19	0.1904
Total	14.12	40	

$$F = \frac{MStreat}{MSres} = \frac{0.1212}{0.1904} = 0.637$$

P = 0.435

$$90\%CI = \bar{\Delta} \pm t\sqrt{EMS\left(\frac{1}{N1}+\frac{1}{N2}\right)}$$

$$90\%CI = (-0.0961) \pm 1.734\sqrt{0.1904\left(\frac{1}{20}+\frac{1}{20}\right)}$$

$$90\%CI = (-0.3354), (0.1432)$$

anti ln

$$90\%CI = 0.7150, 1.1540$$

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; Where EMS = Estimated Mean Square

= Residual Mean Square

$t_{0.1}$, df = 18 = 1.734

4. ln C_{max} after buccal and rectal diazepam administration

(Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	5.182	0.6453	0.1443
Rectal route	20	5.353	0.6577	0.1471

Source of variation	SS	DF	Variance Est (MS)
Between subjects	12.14	19	
Within Subjects	4.279	20	
Treatment	0.2924	1	0.2924
Residual	3.986	19	0.2098
Total	16.42	40	

$$F = \frac{MStreat}{MSres} = \frac{0.2924}{0.2098} = 1.394$$

P = 0.252

$$90\%CI = \bar{\Delta} \pm t\sqrt{EMS\left(\frac{1}{N1} + \frac{1}{N2}\right)}$$

$$90\%CI = (-0.1710) \pm 1.734\sqrt{0.2098\left(\frac{1}{20} + \frac{1}{20}\right)}$$

$$90\%CI = (-0.4222), (0.0802)$$

anti ln

$$90\%CI = 0.6556, 1.0835$$

–

; Where EMS = Estimated Mean Square

= Residual Mean Square

$t_{0.1}$, df = 18 = 1.734

5. ln $AUC_{0\text{-}8hr}$ after buccal and rectal diazepam administration (Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	10.59	0.764	0.1708
Rectal route	20	10.59	0.6992	0.1564

Source of variation	SS	DF	Variance Est (MS)
Between subjects	10.9	19	
Within Subjects	9.485	20	
Treatment	3.08e-05	1	3.085e-05
Residual	9.485	19	0.4992
Total	20.38	40	

$$F = \frac{MStreat}{MSres} = \frac{3.085e-05}{0.4992} = 0.000$$

P = 0.994

$$90\%CI = \bar{\Delta} \pm t\sqrt{EMS\left(\frac{1}{N1}+\frac{1}{N2}\right)}$$

$$90\%CI = (0.0026) \pm 1.734\sqrt{0.4992\left(\frac{1}{20}+\frac{1}{20}\right)}$$

$$90\%CI = (-0.3848), (0.3900)$$

anti ln

$$90\%CI = 0.6806, 1.4770$$

–

; Where EMS = Estimated Mean Square

= Residual Mean Square

$t_{0.1}$, df = 18 = 1.734

6. ln $AUC_{0-\infty}$ after buccal and rectal diazepam administration (Method B : Compartmental analysis)

Group	n	Mean	S.D.	SEM
Buccal route	20	10.99	0.763	0.1706
Rectal route	20	10.92	0.8597	0.1922

Source of variation	SS	DF	Variance Est (MS)
Between subjects	11.78	19	
Within Subjects	13.38	20	
Treatment	0.05739	1	0.05739
Residual	13.32	19	0.701
Total	25.16	40	

$$F = \frac{MStreat}{MSres} = \frac{0.05739}{0.701} = 0.082$$

P = 0.778

$$90\%CI = \bar{\Delta} \pm t\sqrt{EMS\left(\frac{1}{N1}+\frac{1}{N2}\right)}$$

$$90\%CI = (0.0758) \pm 1.734\sqrt{0.701\left(\frac{1}{20}+\frac{1}{20}\right)}$$

$$90\%CI = (-0.3833), (0.5349)$$

anti ln

$$90\%CI = 0.6816, 1.7073$$

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; Where EMS = Estimated Mean Square

= Residual Mean Square

$t_{0.1}$, df = 18 = 1.734

Calculation of Area under the Curve (AUC)

Method A : Non-compartmental analysis

The trapezoidal integral is similar to the AUC in that it is a measure of the area under the curve. It differs for the time period under the data because the trapeoidal integral is calculated based on the data points and not the model parameters. The trapezoidal integral is defined as follow:

$$TRAP_{data} = \sum_{i=2}^{npts} \frac{1}{2}(y_i + y_{i-1})(x_i - x_{i-1})$$

Method B : Compartmental analysis (RSTRIP program)

The area under the curve are defined as follow:

$$AUC = \int_0^{\infty} C(t)dt$$

RSTRIP calcualtes these integrals from the coefficients and rate constant as follow:

$$AUC = \sum_{i=1}^{n} \frac{A_i}{k_i}$$

VITA

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