CHAPTER 2

Literature Review

Carbamazepine (CBZ) is an anticonvulsant agent which has chemical structure related to the tricyclic antidepressant. It is a derivative of iminostilbene with a potent anticonvulsant activity. The structural formula of CBZ is presented in figure 1.[11]

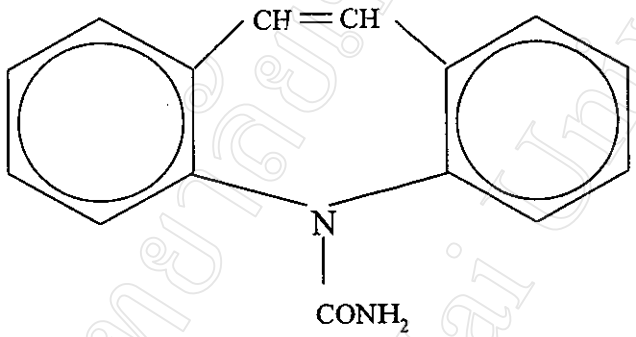

Figure 1 Structural formula of carbamazepine

2.1 Carbamazepine in clinical practice

CBZ was approved in the united states for generalized tonic - clonic seizure, mixed seizure patterns or other partial or generalized seizures agent in 1974. It has been employed since the 1960 for the treatment of trigeminal neuralgia. CBZ can be used for some effective disorder[11] e.g., diabetics insipidus because CBZ has been antidiuretic effect by stimulates antidiuretic hormone release. CBZ also be used for bipolar depression, some in whom lithium carbonate is not effective,[11] tebetic lightning pains, paresthesia associated with Lhermitte's sign,[12] management of alcohol, cocain and benzodiazepine withdrawal.[1]

2.2 Pharmacology of carbamazepine

2.2.1 Mechanisms of action

The mechanisms of anticonvulsant action of CBZ remain unclear. However, it is agreed that CBZ prevents the spreading of epileptic activity probably via the facilitation of inhibitory feedback mechanism in the central nervous system (CNS).[13, 14] This may indirectly dampen the paroxysmal output of epileptogenic foci, while only minimally affecting normal rate of neuronal firing. However, the direct inhibitory effect on epileptic foci cannot be excluded. There is an in vitro evidence that CBZ induces the rate of sustained repetitive firing (SRF) in the cultured spinal cord neurons taken from mouse,[15] and also diminishes excitatory transmission in rat hippocampus at a therapeutic concentration[16] CBZ can also depress the response of trigeminal neurons to an unconditioned maxillary nerve stimulus. Various mechanisms contributed to the above mentioned effects have been proposed, this include:

1) The specific inhibition of neuronal sodium channels in epileptic foci

The high concentrations of CBZ (0.25-1.0 mM) have been demonstrated to inhibit sodium and potassium conductances in the voltage-clamped Myxicola giant axon.[17] And subsequent studies showed that CBZ blocks the binding of batrachoxin and other sodium channel activators (eg. grayanotoxin, veratidine and aconitine) to their receptor sites on sodium channels in rat brain synaptosomes and mouse neuroblastoma cells; and thus preventing the persistant activation of sodium channels by these agents.[18, 19] The concentrations of CBZ required to block the sodium channels in rat brain are closely correlated with the brain levels of CBZ achieved during effective prevention of experimental seizures in rats.[19] Moreover, the voltage-sensitive sodium channels on neuroblastoma cells are not effected by CBZ despite of sodium current is markedly reduced.[20] These finding would support the hypothesis that CBZ specifically inhibition receptor-operated sodium channel which lead to inhibition of neuronal firing in epileptic foci.

2) Alteration of neurotransmitters

Owing to the structural similarity between CBZ and tricyclic antidepressants, the neuronal reuptake of noradrenaline (norepinephrine) and dopamine in cerebellum, hippocampus, cortex and brain stem are blocked by CBZ.[21, 22] It is not yet known whether these effects can be attributable to its antiepileptic properties. But the reduction of catecholamine turnover might be responsible for its antimanic and antipsychotic effects.

The level of other CNS neurotransmitters, namely acetylcholine (Ach) and glutamate may also be altered by CBZ. There is indeed a report of an increased Ach level in rat corpus striatum. This is well correlated to a reduction of choline concentration.[23] And the release of glutamate, an excitatory neurotransmitter, appeared to be inhibited by CBZ at a concentration higher than 10 mcM (2.36 mcg/ml). This concentration of CBZ has no effect on the high affinity uptake of [^{3}H] L-glutamate in the rat hippocampus.[16] But the mechanisms by which CBZ inhibit [^{3}H]L-glutamate release is not yet clearly understood.

3) Stimulation of peripheral-type benzodiazepine receptors

The distribution of the peripheral-type benzodiazepine receptors which was first identified in the kidney, is not confine only peripherally but also found in brain with a high density in olfactory bulb and ependyma.[24-26] CBZ may bind, with a high affinity, to this type of receptors and exerts its anticonvulsant effect.[24] This binding can be prevented by an antagonist of such receptors, Ro 5-4864. The above suggestion is strongly supported by an evidence that pretreatment with Ro 5-4864 blocked the anticonvulsant effect of CBZ but not of diazepam which bind to the central – type benzodiazepine receptors.[24] And the brain concentration of CBZ following therapeutic dose is higher than the circulating level (1.4-1.6 times).[27] This may support the suggestion that the brain is the important site of action of CBZ.

4) Effects on adenosine receptor

There is similarity between the effects of CBZ and adenosine on the CNS, i.e. sedative, hypnotic and anticonvulant.[28-32] Thus, there was a suggestion that CBZ may exert its anticonvulsant action by activating adenosine receptors. But the in vitro binding studies indicated that CBZ may act as a partial agonist.[33] Indeed CBZ, at the therapeutic concentration, could inhibit the binding of adenosine analogs, i.e., [^{3}H] L-N^6-phenyl-isopropyl adenosine and [^{3}H] cyclohexyladenosine; to adenosine receptor at synaptosomal membrane.[34, 35] And CBZ has also been shown to inhibit adenosine-stimulated adenylate cyclase.[36] However, the correlation between the anticonvulsant activity cannot be demonstrated.[36] Yet, an upregulation of the number of adenosine receptors has been proposed to be the mechanism of anticonvulsant action of CBZ.[37] This suggestion is, however, not widely accepted since more recent studies indicated that CBZ acts as an antagonist of adenosine receptors rather than an agonist.[38, 39]

2.2.2 Pharmacokinetics

The oral bioavailability of the drug is more than 70%.[40] Absorption of CBZ from gastro-intestinal tract is slow and variable, but almost complete absorbed.[2]

Volume of distribution of CBZ is about 1 L/kg [41] and after long term monotherapy the volume of distribution are in the same of single dose.[42] CBZ is a neutral and lipophilic agent and the unbound plasma concentrations of CBZ are equilibrium with those in saliva[17] and cerebrospinal fluid.[11, 44]

Approximately, 70%-80% CBZ is bounded to plasma proteins,[40] albumin is a major site of CBZ binding.[45] The reported mean values for the free fraction of drug in plasma range from 24% to 28%[7,46] while those of carbamazepine-epoxide and trans-10, 11-dihydroxy-10,11 dihydro-carbamazepine (CBZ-H) are about 40 % and 60% respectively.[47, 48] There was no significant difference in binding capacity between plasma from patients with renal disease and that from normal subjects. However, the plasma from patients with hepatic disease bound a slightly lower percentage of CBZ than did normal plasma ($p < 0.05$).[46]

CBZ is metabolised in the liver[41] by hepatic microsomal cytochrome P-450 isoenzymes (P450 IIB or P450IIC subfamily).[47, 49]

A major metabolite is CBZ-10, 11-epoxide (CBZ-E),[41] an active metabolite, CBZ-E is almost completely converted by epoxide-hydrolase to be an inactive metabolite, CBZ-H, before excretion in the urine through glomerular filtration [41, 50] by 20-30% of the dose (figure 2).[51] Only 2% or less appear in the urine as unchanged drug [43] and about 28% of the dose is lost in feces in the form of unchanged CBZ.[2] Moreover, CBZ was excreted in the breast milk may reach 60% of maternal plasma concentration.[2] And it can distribute pass through the placenta.[40]

In pregnancy the level of CBZ decrease, while the epoxide metabolite increase[40] since the clearance of the drug increase in pregnancy.[52]

After single oral doses of CBZ, the absorption is fairly complete and the elimination half-life is about 35 hours (range 18 to 65 hours). During multiple dosing or long term therapy the half-life decreased to 10 – 20 hours.[11, 40] Because of its autoinduction activity.

This autoinduction of CBZ metabolism is already occurred during the first dose of the drug and seem to be complete within one month of treatment,[53, 54] which can lead to a 3 times increase in the clearance (figure 3) [54] and the autoinduction also disappeared rapidly after stopping the treatment (figure 4).[53]

The extent of autoinduction differs greatly among patients, making dosage requirements highly variable. The maintenance dose of CBZ may need to be increase during the first few months of treatment in order to maintain the CBZ concentration within the range achieved during early therapy.[52]

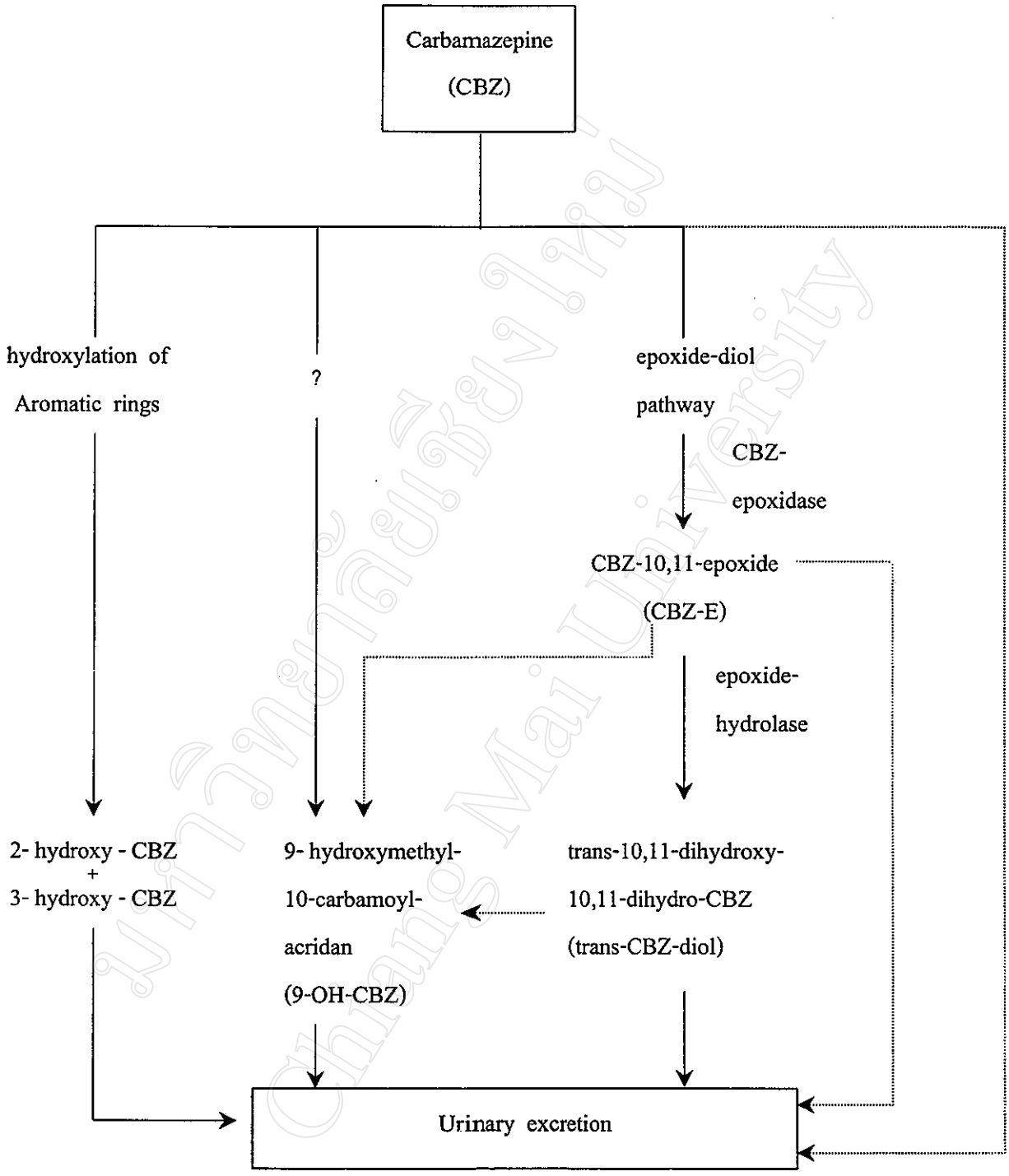

Figure 2 Major pathways of metabolism of carbamazepine in man

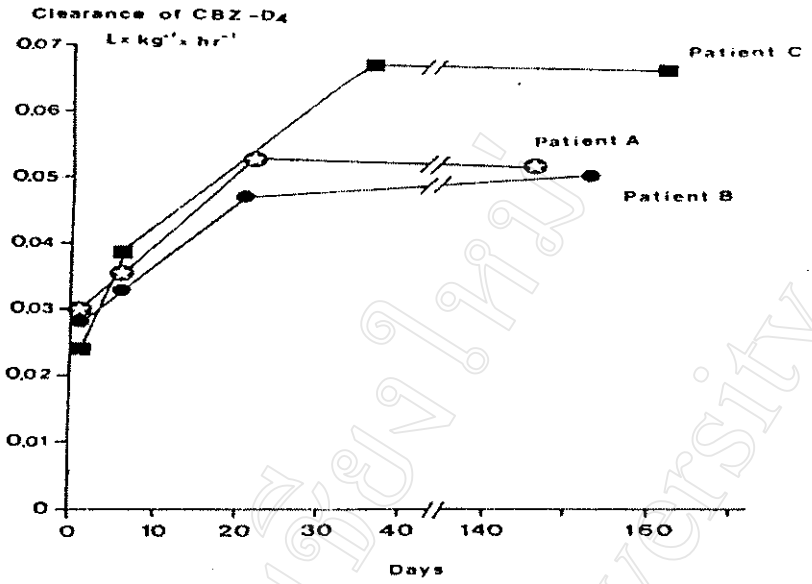

Figure 3 Plasma clearance carbamazepine (CBZ)-D_4 when given as a single oral dose at different times before and during maintenance CBZ treatment in three patients with recently discovered epilepsy. Multiple dosing was started on day 5.

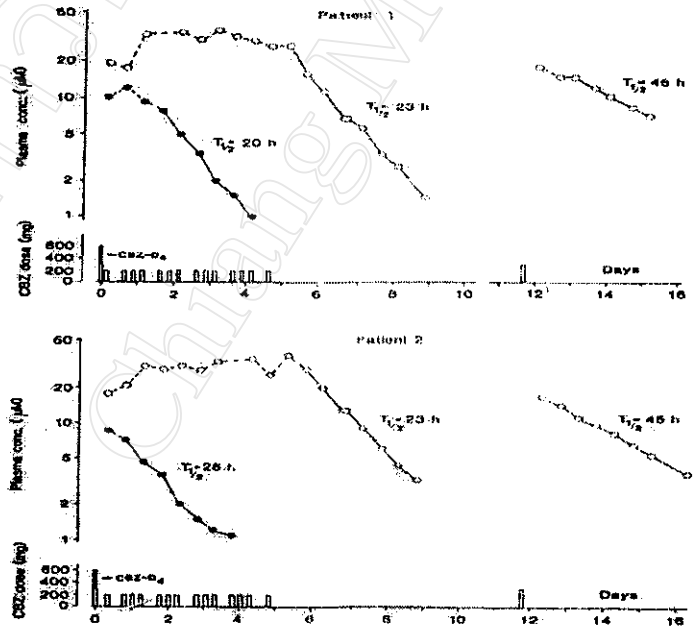

Figure 4 Plasma concentrations of CBZ (○) and CBZ-D4 (●) in 2 alcholic patients, who were treated with CBZ during the withdrawal period.

2.3 Drug interactions

Because of CBZ widespread and long term use, it is frequently prescribed in combination with other drugs, leading to the possibility of drug interactions.

The most important interactions affecting CBZ pharmacokinetics are those resulting in induction or inhibition of its metabolism. The potential for clinically important drug interaction exists because may induce the hepatic metabolism of other drugs or, conversely, other drugs may induce or inhibit the metabolism of CBZ.

Valproic acid,[47] phenytoin, phenobarbital,[40, 42, 55] primidone,[42, 56] ethosuximide, methsuximide,[42] felbamate[57, 58] increase metabolic clearance of CBZ and reduce plasma CBZ concentrations to a clinically important extent. Plasma CBZ concentration may be decreased by some cytostatic drugs such as doxorubicin[59] and cisplatin[60] and by activated charcoal,[56] thereby reduce absorption. Activated charcoal also stimulates the elimination of CBZ, presumably by sequestering the enterically secreted drug.[59]

Inhibition of CBZ metabolism and elevation of plasma CBZ concentration can be caused by fluoxetine,[61, 62] cimetidine,[63] acetazolamide,[64] verapamil,[65, 66] diltiazem,[66] Triacetyl-oleandomycin, erythromycin,[56, 67] clonazepam, propoxyphene,[56] isoniazid, danazol.[59]

Valproic acid (sodium valproate)[47] and verapamil[59] have been reported to displace CBZ from plasma protein binding site but without clinical significance.

CBZ increase level of topiramate, lithium,[68] phenytoin[69] but the metabolism of many other drugs such as clobazam,[70] warfarin,[71, 72] dophenylhydantoin,[71] cyclosporine,[73] oral contraceptive pill,[56] doxycycline, clonazepam,[74] felbamate, haloperidol, neuromuscular blocking agents, several tricyclic antidepressants, lamotrigine[59] can also be induced by CBZ. CBZ may both induce and inhibit the biotransformation of phenytoin and the effect of CBZ on primidone metabolism a significant increase in plasma concentration of metabolically derived phenobarbital.[59]

Thus, dosage adjustment and monitoring the plasma level of each drug are essential for obtaining the good control of seizure with minimum adverse effects of the combination drugs.

2.4 Side effects

The common side effects included drowsiness, loss of appetite, ataxia, dizziness, loss of accommodation, and occasionally nausea but no vomiting, bone marrow toxicity, and seizure can be induced by anticonvulsants: this phenomenon has been reported following therapy with CBZ.[75-77]

Over half (54%) of saliva CBZ concentrations above the therapeutic range were associated with adverse effects.[4] In another report, increased CBZ concentrations in the saliva have been found to be significantly associated ($p < 0.01$) with impaired adaptive tracking, smooth and saccadic eye movements and increase heart rate and plasma concentrations were associated with impaired eye movements and body sway.[78]

Most of the adverse effects of CBZ are associated with the CNS: dizziness, blurred vision, nausea, lethargy and drowsiness which is correlate with the peak absorption of the drug and occur at a predictable drug concentration in blood. Because the time to peak absorption varies from patient to patient, it may be necessary to collect a blood sample at the time the adverse effects are occurring. These adverse effects may be minimized by slowly increasing the dosage.[79] Two dose per day are appropriate in most cases, but some patients may benefit from more frequent dosing to avoid side effect[40] by dividing the daily dosage into unequal amounts and giving the largest dose at bedtime, many of the troublesome CNS depressant effects may be minimized in the bedtime.[79]

2.5 Therapeutic drug monitoring of anticonvulsants

Therapeutic drug monitoring of anticonvulsants should be done according to these following conditions :

1. Poor seizure control.[75]
2. When drug toxicity is suspected.[75, 80]
3. Suspected gross noncompliance.[75]
4. Status epilepticus.[75]
5. At 2-4 weeks after the initiation of the therapy.[75, 80]

6. Modification of concomitant drug therapy (addition, dose change, discontinuation).[75]

7. Intercurrent illness or a change in physiological state (pregnancy).[75, 80]

8. When seizure control has been attained without adverse effects.[80]

9. When seizure recur.[80]

10. When anticonvulsant therapy is about to be withdrawn.[80]

11. When drug interactions are suspected.[4]

12. If the therapeutic concentration range of the drug involved is narrow.[81]

The interindividual variation in the capacity to hydrolize CBZ is marked, hence, monitoring CBZ plasma level has become routine aid for individualization of dosage.[50]

Interestingly, many clinicians in Thailand find that some patients whose epilepsy is well controlled with a proprietary brand of CBZ develop frequent seizure after change to a new generic formulation.[82] This support the use of therapeutic drug monitoring of CBZ.

From many previous reports, suggested that saliva offered a convenient noninvasive alternative to blood for monitoring CBZ therapy [7] in whom venipuncture is not always simple eg. pediatric patients, geriatric patients, obese patients , patients who fear having blood drawn, other who simply have poor venous access.[4-6]

2.6 Salivary therapeutic drug monitoring for anticonvulsants[4]

The first attempt to examine components in saliva as a dignostic tool occurred in the early years of this century. Saliva analysis has provided valuable information for both clinician and investigator in various clinical situations. TDM using blood concentration of anticonvulsant drugs has been used for around for 35 years in clinical practice to aid the management of patients with epilepsy. During the past 30 years, there has also been great interest in the use of saliva for TDM of anticonvulsant drugs. The management of saliva concentrations of anticonvulsant drugs has been applied to pharmacokinetic and pharmacodynamic studies, metabolic studies and for TDM in a variety of seizure disorder.[81, 83, 84]

TDM of anticonvulsant drugs received early attention because: (i) these agents are used long term; (ii) they have a therapeutic range with a readily discernible and temporally coupled pharmacodynamic end-point (i.e. seizure control); (iii) they show marked inter and intra individual dosage requirements; and (iv) seizure control is improved when the drug and active metabolites are measured and the data used appropriately.

Numerous investigators have suggested that saliva can serve as an alternative body fluid for analysis in TDM since a useful relationship exists between the saliva and blood concentrations of most of the commonly used anticonvulsant drugs. The relative value of saliva sampling as opposed to blood, may vary in different setting.[6] The advantages and disadvantages of using saliva samples in TDM of anticonvulsant drugs are list in table 1.

Table 1 Advantages and disadvantages of using samples for therapeutic drug monitoring

Advantages	Disadvantages
- Collection of saliva is simple and noninvasive - Procedure may be cheaper than blood drawing - Does not require the expertise of drawing blood - Preferred by children and their parents - Less stress, fear and discomfort - Avoids complications of infection and thrombosis[85] - Easier to obtain multiple samples - Results are closer to free drug concentrations in most cases	- Potential contamination - Insufficient volume of saliva - Difficulty in pipetting due to the viscosity of saliva - Need standardized saliva collecting procedure - Possible interference by citric acid in enzymatic immunoassay technique assay - Unresolved chromatographic peak in saliva sample - The concentration of some anticonvulsant drugs is low in saliva - Many factors may influence saliva drug concentrations

Particular advantages include the following.

- Saliva can be collected with minimal patient discomfort and can easily be obtained on multiple occasion.

- Frequent measurement of the saliva concentrations of anticonvulsant drugs appears to be a promising and inexpensive adjunct to the investigation and management of certain problems in patients with epilepsy.

- Saliva may be of special interest in paediatric patients as its sampling is convenient, painless and noninvasive.

However, this simple and noninvasive method has not gained adequate acceptance in clinical practice.

Several recent developments provide renewed impetus for the use of saliva sampling in the TDM of anticonvulsant drugs.[6, 86, 87] First, more information is available to better understand the pharmacokinetic principles of drug distribution in saliva. Secondly, over the past decade there has been an increased emphasis on determination of free drug concentrations. Saliva concentration determination may provide a less expensive means of estimating free drug concentrations. Thirdly, new technologies have markedly improved the level of sensitivity and selectivity of analytical methods for the detection and separation of anticonvulsant drugs.[87, 88]

2.6.1 Physiology of the saliva glands

Saliva is secreated by the 3 major paired salivary gland (the parotid, sublingual and submaxillary glands). The blood supply to these glands is provided by the external carotid artery. In humans, secretion from the salivary glands occurs in response to neurotransmitter stimulation. The salivary glands are innervated by both sympathetic and parasympathetic nerves. Generally, sympathetic stimulation via norepinephrine (noradrenaline) leads to high levels of protein secretion, whereas high rates of fluid output occur in response to parasympathetic stimulation via acetylcholine. Approximately 1200 ml of saliva is secreted each day.

Resting saliva is hypotonic and contains small amount of proteins, sodium, potassium and other solutes. The detailed composition of saliva in healthy adults can be found elsewhere.

Resting salivary flow rates demonstrate a circadian rhythm. This can also result in alterations in salivary pH (the pH of saliva can range from 5.8 to 7.8), with the higher pH exhibited upon increased secretion because of an increase in bicarbonate content.

2.6.2 Pharmacokinetic principles of drug distribution in saliva

Saliva serves as a unique compartment for the distribution of drugs. Many basic principles of drug distribution into physiological compartments apply to the salivary distribution of drugs. The transfer of drugs from the blood into saliva depends on the physicochemical properties of the compounds, such as molecular size, lipid solubility, pKa and protein binding. The main physiological factors influencing drug distribution into the saliva are the salivary pH, flow rate and any existing pathophysiology of the oral cavity. These factors have been discussed in detail elsewhere.

Most drug pass from the blood to saliva by simple diffusion along the phospholipid bilayer of acinar cells, which requires that they are lipid soluble, non-ionised and not bound to proteins. The membrane transfer functionally occurs in both directions, and the drug diffuses and equilibrates according to the concentration gradient. An active unidirectional process may be present for some drugs.

The saliva flow rate is one of the most significant factors in determining the concentrations of solutes in saliva. When saliva secretion is stimulated, as compared with the resting state, the contact time is reduced and the processes of tubular reabsorption and secretion play a lesser role upon the final solute concentration. The salivary flow rate has a variable effect on the saliva concentration of different drugs. A rapid flow rate narrows the variability in saliva: plasma concentration ratios of phenytoin and lowers the concentration of primidone in saliva, but only has a minor effect on the saliva: plasma ratios of carbamazepine and valproic acid.[6]

Salivary pH is the chief variable determining the concentration of ionisable drugs in saliva. For an acidic or basic drug, the degree of ionization in the saliva and blood is highly dependent on the pKa of the drug and the pH of the environment. With acidic, largely ionized,

drugs the saliva: plasma ratio decreases with rising pH. The reverse situation occurs with the basic drugs. Since saliva is usually more acidic than plasma, the saliva: plasma ratio is equal to or less than unity for acidic and low protein binding drugs (such as phenobarbital and phenytoin), and is equal to or greater than unity for a basic drug. The saliva: plasma ratio is equal to unity for neutral and non protein binding drugs. The theoretical saliva: plasma ratio can be estimated from equations derived from the Henderson-Hasselbach equation, as demonstrated first by Matin et al.[89]

For acid drugs $C_{usal}/C_{up} = (1+ 10^{pHs-pKa}) / (1+10^{pHp-pKa})$

For basic drugs $C_{usal}/C_{up} = (1+ 10^{pKa-pHs}) / (1+10^{pKa-pHp})$

Where C_{usal} = the concentration of unbound drug in saliva; C_{up} = the concentration of unbound drug in plasma; pH_s= pH of saliva; pH_p= pH of plasma; pKa = pKa value for the drug. The greatest utility for STDM is when $C_{usal}= C_{sal}$, we consider the C_{sal}/C_{up} ratio to be equivalent to the C_{usal}/C_{up} ratio.[89]

Among the commonly used anticonvulsant drugs, phenobarbital is the drug with salivary concentrations that may be influenced by the pH of the environment.[6]

Drug protein binding is another factor determining the saliva: plasma ratio. Plasma contains both free and bound drug, whereas saliva generally contains only the unbound drug. It is a fundamental principle in pharmacology that only the free drug is able to interact with the intracellular receptor sites and exert an effect. Since the diffusion of the drug between blood and saliva is limited to the unbound fraction of drug, significant changes of drug binding, in either plasma or saliva, will alter the free drug concentration within these compartments. The causes of variability in free fraction of drug concentration include:

- saturation of binding
- pathophysiological changes in α1-acid glycoprotein concentration
- pathophysiological changes in albumin concentration
- pathophysiologically induced competitive binding.

Criteria that were developed for monitoring the free, rather than total, concentrations of anticonvulsant drugs include extensive and variable binding to plasma proteins. Phenytoin and valproic acid belong to this category, whereas ethosuximide and primidone are not significantly protein bound. The free fractions of valproic acid and phenytoin may increase because of saturable binding and competitive binding.

One problem with the interpretation of saliva: plasma ratios is their inconstancy during the 3 pharmacokinetic phases (absorption, distribution and elimination). There are at least 3 reasons for this: (i) the phenomenon of fluctuating arterial-venous differences; (ii) different elimination kinetics from blood and saliva; and (iii) concentration-dependent protein binding.

Although Miles et al.[9] showed inter and intra patient variability for several of the anticonvulsant drugs (mainly because of differences in saliva flow rate and pH), they suggested that, based on the saliva: serum concentration ratio of each of the anticonvulsant drugs, the intraindividual variability of carbamazepine, phenobarbital and phenytoin concentrations in saliva is not a factor that should dissuade clinician from using saliva samples for the TDM of these medications.[9]

2.6.3 Diseases and drugs influencing salivary secretion

Unstimulated slivary flow rate is affected by many factors. Hydration, hyperhydration, exposure to light, olfactory stimuli and even body positioning can influence the flow rate.[9] Several diseases and drug treatments may change salivary flow and/or composition. Decrease salivary secretion may result from diseases directly affecting the salivary glands, such as Sjogren's syndrome or congenital xerostomia. Alteration may also occur secondary to diseases such as cystic fibrosis, systemic lupus erythematosus and Cushing's syndrome.

Anticholinergic drugs may interfere the neural control of salivary function and reduce the secretion of saliva. Reduced salivary flow may also be an adverse effect of numerous drugs such as antidepressants, anticonvulsants and cancer chemotherapy agents.

So far the influence of physiological and pathological causes of salivary gland dysfunction on salivary drug concentrations have not been rigorously examined in a systematic fashion.

2.7 Relationship between plasma-saliva concentration of CBZ and clinical effect

Dose and CBZ saliva levels were significantly related[78, 90] but no correlation with efficacy.[75, 90] Relance and Moreland (1981) found a positive correlation between saliva CBZ concentration and toxicity. They suggested a therapeutic range for CBZ in saliva of 1.2 to 3.5 mg/l.[75]

In contrast, some researchers reported that the relationship between dose and serum level for CBZ is poor [11, 12, 76, 91] because of difference in genetics, age, gender, variable absorption, drug interaction, autoinduction and disease state between individuals.[47, 49, 91] Optimum seizure control usually occurs when plasma CBZ concentration are maintained in the range of 4 – 12 mcg/ml (17 – 51 mcmole).[2]

Anticonvulsant drug levels can be correlated with seizure frequent and the effect of the menstrual cycle on drug levels noted (figure 5). Normal hormonal fluctuations may influence the frequency of seizures, and epilepsy can cause changes in endocrine function. For example, some women will have a marked change in the pattern of their seizure during particular parts of the menstrual cycle (catamenial epilepsy).[88]

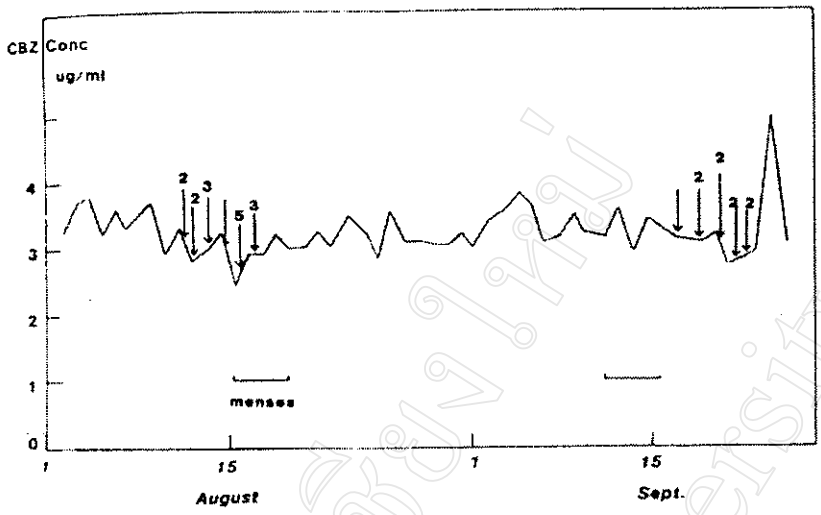

Figure 5 Time course of salivary CBZ levels in a patient receiving 200 mg of the drug twice daily. Arrows indicated days of seizure activity.

Linear relationships between serum/plasma concentrations and saliva concentrations have been reported for CBZ by many investigators. The saliva: plasma total concentration ratios ranged from 0.26 to 0.34 and the correlation coefficients range from 0.84 to 0.99. (Table 2)

The saliva and cerebrospinal fluid CBZ concentration in adults has been reported to be the same amount as free fraction of the drug in adult plasma.[8] In the other hand, CBZ-E has been shown to have anticonvulsant activity in animals, but anticonvulsant activity in human has not been shown.[79] Recent experiments in the squirrel monkey with the use of 14C-CBZ have shown that CBZ-E penetrates into the brain with a lesser extent than CBZ, this due to lower lipid solubility of CBZ-E.[7] The patients who did not response to therapy had higher fractions of metabolites than did responding patients. It is possible that the more polar epoxide diffuses more slowly into the brain and has reduced antiepileptic potency in patients[91] and the ratio between CBZ-E and CBZ in the brain was found to be much lower than the corresponding value in plasma, hence, the therapeutic impact of the epoxide seems to be of minor importance.[7]

Although CBZ-E contributes to the pharmacological and neurotoxic effects of CBZ this metabolite is not routinely measured.[52]

Table 2 Studies of saliva and blood concentrations of CBZ

Investigator	Assay	No. of subject	Saliva type	Subjects	Other drugs	[SCBZ]/ [PCBZ] ratio	Correlation	p-value
Gorodischer et al. 1997 [6]	HPLC	85	NS, S	Aged 1 to 18 years	Phenobarbital, Phenytoin, Valproate Primidone, Clobazam, Nitrazepam, Diazepam, Ethosuximide	NA	0.84	< 0.001
Westenberg HGM et al. 1978 [7]	HPLC	10	NS, S	3 men and 7 women aged 17 to 63 years	Fluphenazine, Perphenazine, Orphenadine, Diazepam, Fluspirilene	0.26 ± 0.01	0.991	< 0.001
Rylance GW et al. 1977 [8]	GLC	15	S	aged 3 to 4.5 years	NA	0.30 ± 0.07	0.87	< 0.001
Miles MV et al. 1991 [9]	FPIA	6	NS	Healthy, male, age 20 to 26 years	NA	0.33 ± 0.02	NA	NA
McAuliffe et al. 1977 [84]	GLC	12	S	NA	NA	0.26 ± 0.24	0.97	NA

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Table 2 (Continued)

Investigator	Assay	No. of subject	Saliva type	Subjects	Other drugs	[SCBZ]/ [PCBZ] ratio	Correlation coefficient	p-value
Rylance GW et al. 1981 [90]	GLC	20	NS	aged 2.1 to 14.3 years	NA	NA	NA	NA
Paxton JW et al. 1980 [92]	EIA	6	NS, S	Healthy , 3 men and 3 women, 23-36 years	Hormonal contraceptives	0.26 ± 0.05	0.872	< 0.001
Mile MV et al. 1990 [93]	FPIA	51	NS	25 men and 27 women age 11.6 ± 5.3	Phenobarbital, Phenytoin, Valproic acid, Primidone, Other	0.33 ± 0.05	0.92	<0.0001
Rosenthal E et al. 1995 [94]	FPIA	61	NS, S	30 boys and 31 girls, aged 5-16 years	Ethosuximide, Primidone, Cromoglycate, Clobazam	0.34 ± 0.04	0.89	< 0.001

NA = not available; EIA = enzyme immunoassay; FPIA = fliorescence polarisation immunoassay; GLC = gas- liquid chromatography; HPLC = high performance liquid chromatography; NS = non-stimulated; S = stimulated.

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2.8 Methodology

Several methods of analysis of CBZ in plasma or serum have been described involving immunologically based technique groups were enzyme immunoassay (EIA),[95] substrate-labeled fluorescence immunoassay (FIA),[91] fluorescence polarization immunoassay (FPIA)[9, 91, 96] and chromatographic technique group were gas-liquid chromatography (GLC)[8, 10, 76, 95] and high-performance liquid chromatography (HPLC).[6, 40, 48, 87, 88, 95, 97, 98] The result of comparison of CBZ measurements by the three different method (HPLC, GLC, EIA), there was a very close correlation coefficient in excess of 0.97.[95]

The FPIA was significantly were precise than other techniques but GLC was least precise for CBZ.[96] The major problem for GLC is the decomposition of the CBZ to iminostilbene and 9-methylacridine.[99] However, immunoassay techniques for CBZ determination had a 16-21% cross-reactivity with CBZ-E.[94]

HPLC is a rapid, accurate, sensitive, precise method for simultaneous quantitation of CBZ.[48, 87, 88, 95, 97, 100] Moreover, the cost of this method is lower than the others and it can be used to determine CBZ with other antiepileptic drugs such as phenytoin, valproic acid, phenobarbital, ethosuximide, etc.[101] Therefore the present study chooses HPLC which is the most suitable method for determination of CBZ in plasma and saliva.