Therapeutic Drug Monitoring of Antiepileptic Drugs by Use of Saliva

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Abstract: Blood (serum/plasma) antiepileptic drug (AED) therapeutic drug monitoring (TDM) has proven to be an invaluable surrogate marker for individualizing and optimizing the drug management of patients with epilepsy. Since 1989, there has been an exponential increase in AEDs with 23 currently licensed for clinical use, and recently, there has been renewed and extensive interest in the use of saliva as an alternative matrix for AED TDM. The advantages of saliva include the fact that for many AEDs it reflects the free (pharmacologically active) concentration in serum; it is readily sampled, can be sampled repetitively, and sampling is noninvasive; does not require the expertise of a phlebotomist; and is preferred by many patients, particularly children and the elderly. For each AED, this review summarizes the key pharmacokinetic characteristics relevant to the practice of TDM, discusses the use of other biological matrices with particular emphasis on saliva and the evidence that saliva concentration reflects those in serum. Also discussed are the indications for salivary AED TDM, the key factors to consider when saliva sampling is to be undertaken, and finally, a practical protocol is described so as to enable AED TDM to be applied optimally and effectively in the clinical setting. Overall, there is compelling evidence that salivary TDM can be usefully applied so as to optimize the treatment of epilepsy with carbamazepine, clobazam, ethosuximide, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytoin, primidone, topiramate, and zonisamide. Salivary TDM of valproic acid is probably not helpful, whereas for clonazepam, eslicarbazepine acetate, felbamate, pregabalin, retigabine, rufinamide, stiripentol, tiagabine, and vigabatrin, the data are sparse or nonexistent.

Key Words: saliva, antiepileptic drugs, therapeutic drug monitoring, practical protocol for saliva antiepileptic drug monitoring

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INTRODUCTION

Measuring antiepileptic drugs (AEDs) in serum or plasma as an aid to personalizing drug therapy is now a well-established practice in the treatment of epilepsy, and guidelines are published that indicate the particular features of epilepsy and the properties of AEDs that make the practice so beneficial.[1] The goal of AED therapeutic drug monitoring (TDM) is to optimize a patient's clinical outcome by supporting the management of their medication regimen with the assistance of measured drug concentrations/levels. The reason why TDM has emerged as an important adjunct to treatment with the AEDs arises from the fact that for an individual patient identifying the optimal dose on clinical grounds alone can be difficult and there are many reasons for this including the following: (1) AED treatment is prophylactic and, because seizures occur at irregular intervals, it is often difficult to ascertain whether the prescribed dose will be sufficient to produce long-term seizure control; (2) clinical symptoms and signs of toxicity are not always readily detectable; (3) the correlation between AED serum concentration and the clinical effects is much better than that between the dose and effect; and (4) there are no direct laboratory markers for clinical efficacy or AED toxicity.

Although reasonably well-defined reference ranges (target ranges) have been established for most of the AEDs,[1–4] one size does not fit all, and individual differences in the nature and severity of epilepsy result in the effective, nontoxic AED concentration being extremely variable; seizures in some patients can be well managed at serum concentrations below the target range, whereas other patients need and tolerate drug concentration in excess of the range.[1,2] Furthermore, many factors cause unpredictable and sometimes large differences between individuals in pharmacokinetics and disposition of AEDs, which makes it impossible to predict the optimum dose for a particular patient and measuring a serum concentration will often be the most effective way to guide treatment. Indeed, the concept of the "individual therapeutic range" has been championed as the ideal practice parameter for bespoke AED therapy,[1] and a similar approach has recently been advocated for psychiatric drug therapy.[5]

Although AED TDM for the treatment of epilepsy was initially developed and validated for the few drugs that were available during the 1960s–1980s, a further 17 drugs have been introduced since 1989 some of which are also effective for managing other neurological disorders (Table 1). The clinical trials of investigational AEDs are undertaken primarily to establish safety, ascertain pharmacokinetics, and dosage range, their drug–drug interaction profiles, their efficacy over placebo, and to identify acute adverse effects.[6] These are the

TABLE 1. Introduction of Antiepileptic Drugs in the United Kingdom*

Drug	Year of Introduction
Phenobarbital	1912
Phenytoin	1938
Primidone	1952
Ethosuximide	1960
Carbamazepine	1963
Valproate	1974
Clonazepam	1974
Clobazam	1982
Vigabatrin	1989
Lamotrigine	1991
Gabapentin	1993
Felbamate	1993
Topiramate	1995
Tiagabine	1998
Oxcarbazepine	2000
Levetiracetam	2000
Pregabalin	2004
Zonisamide	2005
Rufinamide	2007
Stiripentol	2007
Lacosamide	2008
Eslicarbazepine acetate	2009
Retigabine	2011

*Although in general the order of drug introduction is similar in Europe and the United States.

TABLE 2. Indications for AED Therapeutic Drug Monitoring

	Indication	Comment
1	After initialization of AED treatment or after dose adjustment	This allows the pursuance of a preselected reference range for the individual patient.
2	Upon achievement of optimum desired clinical response	Seizure freedom is the optimum outcome, but for many patients, optimum seizure control with minimal adverse effects is more readily achieved. The "individual therapeutic range" can be established.
3	To determine the magnitude of a dose change	This is particularly important for AEDs that show dose-dependent pharmacokinetics (eg, phenytoin, carbamazepine, valproate, gabapentin, stiripentol, and rufinamide).
4	When toxicity is difficult to differentially diagnose or when toxicity is difficult to assess clinically	Concentration-related AED toxicity is more readily identified and is particularly helpful when young children or patients with mental disability are being evaluated.
5	When seizures persist despite the prescribing of an adequate/typical dosage	This may identify a fast metabolizer or a patient that is noncomplying with their AED medication.
6	When pharmacokinetic variability is expected	This is a significant category of patients and includes children, the elderly, during pregnancy, hepatic disease, renal disease, various pathologies, postsurgery, and drug–drug interactions.
7	When a formulation change has occurred	This includes brand-to-generic and generic-to-generic switches.
8	The clinical response has unexpectantly changed	The cause of the change could be readily identified as it could be the consequence of many reasons.
9	Poor compliance suspected	Recent noncompliance can be readily identified. However, long-term compliance or variable compliance cannot be identified.

characteristics that must be documented to achieve regulatory approval. Although serum concentration measurements of the investigational AEDs are undertaken (often retrospectively) during the clinical trial process, information regarding the serum concentration to effect/toxicity interrelationship is rarely evaluated at this time. Although the range of serum concentrations determined at the dose ranges investigated during clinical trials of a new AED give some useful information regarding a putative reference range, the correlation with clinical effect is rarely evaluated. Nevertheless, this information can prove useful clinically, particularly when it is remembered that serum concentration measurements should be used in the context of the patient's clinical presentation (ie, treat the patient not the serum concentration). The indications for AED TDM are shown in Table 2.

The aim of this review is to discuss the potential use of saliva as a matrix to undertake AED TDM. First, the advantages and disadvantages of using various biological matrices with particular emphasis on saliva will be reviewed. Second, for each AED, the key pharmacokinetic characteristics relevant to the practice of TDM are presented along with the evidence that saliva concentrations reflect those in serum. Third, indications for salivary AED TDM are emphasized along with the key factors to consider when saliva sampling is to be undertaken. Lastly, and finally, a practical protocol is described so as to enable AEDS TDM to be applied optimally and effectively in the clinical setting. Search strategy and selection criteria: This review is based on published articles and searches in PubMed and Google Scholar up to April 2012, in addition to references from relevant articles. Primary sources were preferred, but abstracts are included where no subsequent peer reviewed article was published. Review articles of importance were also used. The search terms included the various AEDs: carbamazepine, clobazam, clonazepam, eslicarbazepine acetate, ethosuximide, felbamate, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytoin, pregabalin, primidone, retigabine, rufinamide, stiripentol, tiagabine, topiramate, valproic acid, vigabatrin, and zonisamide. Also, the terms saliva, hair, cerebrospinal fluid (CSF), tears, dried spot blood, and TDM were searched.

BIOLOGICAL MATRIXES

A variety of biological matrixes have been employed for TDM of AEDs, and these are reviewed in this section. The various matrices have not been validated for all drugs; in particular, some have not been investigated with respect to the newer AEDs. When the final analytical step is chromatographic, any of the matrices requires some form of sample pretreatment/extraction before the final analytical process. If immunoassays are used, they must also be matrix validated; furthermore, immunoassays are not commercially available for all AEDs discussed in this review.

Blood

TDM is normally undertaken in serum or plasma, and these matrixes can be used interchangeably because no differences in AED concentration have been demonstrated between them. Many AEDs are bound to serum proteins, and all analytical methods measure the total serum/plasma concentration [ie, the sum of drug bound to serum protein (usually albumin) and free non–protein bound drug]. Although this parameter will suffice in most clinical settings, it is only the free non–protein bound drug that can cross the blood–brain barrier to equilibrate with brain receptors and produce the pharmacological effect. Therefore, one should ideally measure the free non–protein bound drug concentration, and there are clinical situations where protein binding is disturbed and monitoring the free non–protein bound (pharmacologically effective) drug would be more appropriate. This is particularly true for those AEDs that are extensively protein bound (eg, phenytoin, carbamazepine, and valproate). Settings where protein binding can be impaired include the following:

- *Hypoalbuminemia*: This can occur with renal disease, liver disease, pregnancy, old age, postsurgically, and a range of other pathological conditions (see "Pathological states").
- Conditions in which endogenous protein displacing agents accumulate, for example, uremia.
- Binding displacement by another significantly protein-bound drug.

The extent to which a binding disturbance occurs is unpredictable and may differ from patient to patient and also within the same patient over time. If the free drug concentration increases, the determination of total serum concentration will provide an underestimate of the quantity of non–protein bound drug with therapeutic and toxic effects being observed at total concentrations that are lower than expected.

The 3 most common methodologies that are used to separate the "free" from the "bound" components in serum are equilibrium dialysis, ultrafiltration, and ultracentrifugation, with ultracentrifugation being the method of choice in pathology laboratories.[7–9] However, the ultracentrifugation method is time consuming and labor intensive to perform and therefore more costly and thus saliva, which is a natural ultrafiltrate of serum, is a superior matrix for the measurement of free drug concentrations.

Dried Blood Spot

Dried blood spot applications in TDM were recently reviewed by Edelbroek et al,[10] and the approach has been used for a variety of AEDs including carbamazepine, phenytoin, phenobarbital, oxcarbazepine, lamotrigine, rufinamide, gabapentin, and topiramate.[11–16] The specimens can be collected by a finger prick at an agreed time either by the patients themselves or by a carer. After pricking the finger, a blood spot of adequate size (usually 10 mm) is placed onto an absorbent paper where it dries. The sample is then sent together with an AED sample request form to the laboratory (this can be posted). Upon receipt, the laboratory can punch a standard size disc from the paper (eg, an 8-mm disk would contain approximately 15 μL of blood), and the dried blood spot is then extracted/processed before chromatographic examination.

Advantages of the dried blood spot include the fact that sampling is simple, safe, and can be carried out in a home environment without the need of a trained phlebotomist. Only a small amount of blood is required, and if necessary, a sequence of specimens can be easily collected in 1 day. Specimens can always be collected at the optimum time, and the result can be available for a subsequent clinic visit thus allowing immediate adjustment of medication. Thus, the technique is patient friendly and has a better cost benefit than phlebotomy. Dried blood spot samples are particularly helpful for patients that are difficult to bleed and also when a specimen needs collecting at a particular time so as to ascertain whether or not symptoms (eg, transient toxicity) are drug related.

There are also some disadvantages, for example, the quality of both the blood spot and paper affect the result and some blood spots are not suitable for testing; also, some patients are resistant to finger prick.

Tears

Some AEDs (eg, phenobarbital, carbamazepine, phenytoin, primidone, ethosuximide, and valproic acid) are transported into tears, and the concentration represents the free non–protein bound concentration of the drug in serum.[17–22] Furthermore, tear fluid, especially after stimulation, is more homogeneous and more constant in its composition compared with saliva. However, collection of tear samples is rather cumbersome as it involves the use of a capillary tube and is considered by many patients to be invasive because they cannot readily produce tears particularly the quantity (100 μL) currently required for drug analysis. Although it is stated that lacrimation is frequent in children, in adults, brisk tearing has been provoked by smoking and/or the sniffing of formaldehyde—the latter methods being rather undesirable.

Sweat

Some AEDs are secreted into sweat and Parnas et al[23] reported that phenytoin, carbamazepine, and phenobarbital were all present in sweat and that phenytoin concentrations corresponded to the free non–protein bound fraction in serum and were independent of sweat flow. However, phenobarbital sweat concentration increased with sweat flow. Phenobarbital has also been determined in sweat collected into a sweat patch where its presence is easily demonstrated but this procedure would only be useful for documenting drug use over the period during which the patch was applied.[24] At present, sweat is not a very useful matrix for TDM because of the practicalities

associated with flowing sweat collection and/or the interpretation of the concentration if collected into a patch.

Cerebrospinal Fluid

Measurement of CSF concentrations of neuroactive drugs, including AEDs, is important because such concentrations are considered to reflect those occurring in the brain, which result in the pharmacological effect of the drug (eg, anticonvulsant or adverse effects). Furthermore, CSF concentrations are considered to reflect the free, non–protein bound serum concentration.

The transfer of the first-generation AEDs (carbamazepine, phenytoin, phenobarbital, and valproate) into CSF has been well studied, and the CSF concentration generally reflects their free non–protein bound concentration in serum.[20,21,25–28] More recently, some of the second-generation drugs, for example, gabapentin, oxcarbazepine, lamotrigine, levetiracetam, vigabatrin, and topiramate[29–33] and third-generation AEDs, for example, eslicarbazepine acetate[34] have been investigated.

Because for many AEDs the CSF concentration reflects the free non–protein bound drug concentration, this would probably be a meaningful matrix for TDM purposes; however, for some AEDs CSF does not reflect free serum concentration for example, gabapentin and pregabalin.[30–33] This lack of correlation is probably due to the mechanism by which gabapentin and pregabalin are distributed throughout the body, that is, the L-amino acid transporter, which is saturable so that transportation does not occur linearly. In the case of pregabalin, the CSF/serum area under the concentration versus time curve 0–24 hours ratio was 0.098 ± 0.016.[33] The invasive procedure (ie, lumber puncture) needed to collect a CSF samples negates the practical use of CSF for AED TDM.

Hair

The root of every growing hair is constantly exposed to any drug that is circulating in the blood. The drug(s) are thus sequestered into the hair structure and because head hair grows at approximately 1 cm/mo, if only a single drug exposure occurred the portion of hair containing the drug would emerge from the scalp after 6/7 days. This small section would then grow away from the scalp, and by sampling the hair in 1-cm lengths, it is possible to assay the drug concentration in the hair sections and pinpoint the time of exposure. However, in patients prescribed maintenance drug treatment, provided that they are adherent, the hair root will be exposed to a constant, steady-state drug concentration in blood and this would be reflected by a constant concentration of the drug along the hair shaft. Hair can thus be used to record the history of drug exposure and ascertain variable and intermittent compliance. The AEDs that have been reported to be transported into hair include carbamazepine, phenytoin, valproic acid, oxcarbazepine, and lamotrigine.[35–39]

Although hair analysis would not, therefore be helpful for day to day TDM, these principles have been applied to demonstrate compliance with carbamazepine and oxcarbazepine treatment in adult patients,[39–41] compliance of carbamazepine and lamotrigine during pregnancy[38] and to differentiate between chronic and acute carbamazepine intoxication.[42]

The disadvantages of hair analyses for AED TDM include the fact that many factors impact on the amount of drug deposited into hair, for example, melanin content, and whether cosmetic hair treatments, for example, bleaching, dying, etc, remove drugs that are bound into the hair structure and thus cause interpatient concentrations to vary significantly. Also, because drugs are incorporated into the hair structure, the analytical process requires an initial digestion to release them before the chosen method of detection and quantification. Finally, although hair sampling is considered not to be invasive, it can be so for those patients who have little hair or are indeed bald in which case a nonhead hair sample might be required.

Saliva

Saliva was initially investigated as an alternative biological fluid for TDM of AEDs during the 1970s; the most studied drugs are phenytoin, phenobarbital, and carbamazepine.[43–47] Saliva is once again emerging as a biological fluid that is valuable for AED TDM and has started to be more widely used again because it is associated with numerous advantages over blood/serum (Table 3). Of particular advantage is that the concentration in saliva generally reflects the free non–protein bound pharmacologically active component in serum; saliva is easier to collect than blood and patients prefer saliva sampling over blood sampling. Furthermore, the standard analytical methods can invariably be easily adapted to accept saliva specimens.

INDIVIDUAL AEDS

In the following section, the AEDs will be reviewed in alphabetical order with regards to their clinical indications, key pharmacokinetic characteristics in relation to TDM in general and salivary TDM in particular (Table 4), along with the available evidence regarding the usefulness of saliva as a matrix for undertaking AED TDM.

Carbamazepine

Clinical Indications

Carbamazepine is a first-line drug for the treatment of partial and secondarily generalized tonic–clonic seizures and primary generalized tonic–clonic seizures. It is also the drug of choice in the management of trigeminal neuralgia and in addition is used in the treatment of bipolar disorder that is unresponsive to lithium. Carbamazepine is available in a variety of formulations including, tablets, chewable tablets, liquid oral suspension, suppositories, and extended release tablets and capsules.

Pharmacokinetic Characteristics

Absorption of carbamazepine after oral ingestion is erratic and variable with a bioavailability of 75%–85% and T_{max} values that are formulation dependent.[81] The drug is a powerful inducer of hepatic enzymes, and after initiation of treatment, the pharmacokinetic parameters (half-life and clearance) change considerably due to autoinduction, which generally is complete in about 3 weeks.[82] Protein binding is 75%. Carbamazepine is extensively metabolized in the liver, primarily by CYP3A4 with some contribution by CYP2C8, to carbamazepine–epoxide, which is pharmacologically active, equipotent to the parent drug and accumulates in serum to a

TABLE 3. The Advantages and Disadvantages of Using Saliva for AED Therapeutic Drug Monitoring

Advantages	Comments
Reflects free non–protein bound concentration in blood	This is the ideal concentration measurement in blood as it is that component (pharmacologically relevant) that is accessible to the brain where AEDs have their effect.
Collection is simple and noninvasive	Avoids complications of infection and thrombosis, which can be associated with blood sampling. Useful for patients with needle phobias or difficult veins.
Does not require the expertise of drawing blood	Sampling can be undertaken by spouse, partner, parent, or carer.
Cheaper than drawing blood	No need for a phlebotomist, a nurse, or a doctor to bleed the patient.
Especially useful in patients with disabilities, the elderly, and in children	Preferred by patients, parents, and carers.
Less stress, fear, and discomfort	Patients are more amenable to providing multiple samples.
Can be readily undertaken in the home environment	Samples can be collected at the "ideal" time (trough and predose) and readily dispatched to the hospital laboratory in advance of the patient's clinic visit.
Disadvantages	**Comments**
Spurious results due to contamination (drug residues in the mouth or leakage of drug-rich exudate, eg, patients with gingivitis)	This can be avoided by sampling just before AED ingestion (at trough), after the mouth is rinsed or after a few hours have elapsed since drug ingestion.
Saliva volume insufficient	This can be overcome in the laboratory by adding distilled water.
Difficulty in pipetting due to viscosity of saliva	Sample may need to be rejected and patient resampled.
AED concentration is low	Analytical methods need to be specifically developed so as to be able to measure the anticipated low concentrations.
Sampling may be unacceptable	Some patients may refuse sampling, although in the authors' experience this has yet to happen.

variable extent. Carbamazepine–epoxide is subsequently metabolized, by epoxide hydrolase, to a pharmacologically inactive 10,11-diol, which is eliminated partly unchanged and partly as a glucuronide conjugate.[83] Protein binding of carbamazepine–epoxide is 50%–60%. The serum elimination half-life ($t_{1/2}$) of carbamazepine in adults is 8–20 hours, whereas that of carbamazepine–epoxide is approximately 34 hours. Carbamazepine is subject to many drug-drug pharmacokinetic interactions because it is an inducer of hepatic metabolism and also its own metabolism is readily inhibited or induced—consequently there are large differences between individuals in the dose to serum concentration relationship. In addition, although there is a broad range of serum concentrations associated with an optimum effect, there is considerable interpatient variability in the concentration of carbamazepine that is associated with an optimal therapeutic response (which may in part be due to the variation in carbamazepine–epoxide concentration). The fact that the dose to serum concentration relationship of carbamazepine and carbamazepine–epoxide is nonlinear, and thus unpredictable, is the main reason why their monitoring is useful. The current reference range for carbamazepine in serum is 4–12 mg/L (17–51 μmole/L), whereas carbamazepine–epoxide concentrations are generally 5%–15% of the parent drug with a reference range of up to 2.3 mg/L (up to 9 μmole/L).[1]

Saliva TDM for Carbamazepine

There have been many studies investigating the distribution of carbamazepine and carbamazepine epoxide in saliva and several have evaluated the relationship between salivary concentration and the free, pharmacologically active, non–protein bound concentration in serum of both adults and children with epilepsy.[48–54,56,73,84–95]

The salivary concentration of carbamazepine is similar to the free, non–protein bound concentration in serum with mean saliva/serum total carbamazepine concentration ratios ranging 0.26–0.44, whereas the mean saliva/serum-free carbamazepine concentration ratios ranged 1.39–1.44. Indeed, concentrations of carbamazepine in saliva are significantly correlated with both serum total (r^2 = 0.84–0.99) and serum-free carbamazepine concentrations (r^2 = 0.91–0.99). Carbamazepine–epoxide distributes into saliva such that the salivary concentration is similar to the free non–protein bound concentration in serum with mean saliva/serum total carbamazepine–epoxide concentration ratios of 0.31–0.55. Saliva carbamazepine–epoxide concentrations are significantly correlated with both serum total (r^2 = 0.76–0.88) and serum-free (r^2 = 0.75–0.98) carbamazepine–epoxide concentrations. Thus, saliva may be used as an alternative matrix for TDM of both carbamazepine and carbamazepine–epoxide.

Clobazam

Clinical Indications

Clobazam is a 1,5-benzodiazepine drug with marked anticonvulsant properties, which is less sedating than other benzodiazepines. It is licensed for use as adjunctive therapy of partial seizures or generalized seizures in patients above 3 years of age and also for the management of nonconvulsive status epilepticus. It is also prescribed as an anxiolytic. Clobazam is available in tablet and capsule formulations.

Pharmacokinetic Characteristics

Clobazam is rapidly absorbed after oral ingestion with a T_{max} of 1–3 hours and bioavailability of >95%. Its pharmacokinetics is linear and protein binding is 85%. Clobazam is extensively metabolized in the liver, primarily by CYP2C19 and CYP3A4, to *N*-desmethyl clobazam, which is pharmacologically active, accumulates in serum to much higher concentrations than the parent drug, and is responsible for much of the clinical effect.[96] *N*-desmethyl clobazam is subsequently metabolized by CYP2C19 and cleared from serum at a significantly slower rate than the parent drug, with the half-life of clobazam in adults being 10–30 hours, whereas the half-life of *N*-desmethyl clobazam is 36–46 hours. The protein binding of *N*-desmethyl clobazam has not been reported. Clobazam is

TABLE 4. Pharmacokinetic Parameters and Serum Reference Ranges for the Various AEDs Prescribed as Monotherapy to Adults

AED	Time to Steady State (d)	Serum Protein Binding (%)	Half-Life (h)	Reference Range*		Pharmacologically Active Metabolites That Need Monitoring	Saliva Monitoring Validated (Key References)
				Mg/L	Mmole/L		
Carbamazepine	2–4†	75	8–20†	4–12	17–51	Carbamazepine-epoxide‡	[48–53]
Clobazam	7–10§	85	10–30	0.03–0.3	0.1–1.0	*N*-Desmethyl-clobazam	[54,55]
				0.3–3.0¶	1.0–10.5¶		
Clonazepam	3–10	85	17–56	0.02–0.07	0.06–0.22		Not validated
Eslicarbazepine acetate#	3–4	30	13–20	3–35**	12–139**	Eslicarbazepine	Not validated
Ethosuximide	8–12	0	40–60	40–100	283–708		[21,49,50,56–58]
Felbamate	3–5	25	16–22	30–60	126–252		Not validated
Gabapentin	1–2	0	5–9	2–20	12–117		[59,60]
Lacosamide	3	90	13				[61]
Lamotrigine	3–8	55	15–35	2.5–15	10–59		[62–64]
Levetiracetam	1–2	0	6–8	12–46	70–270		[65–67]
Oxcarbazepine††	2–3	40	8–15	3–35	12–139	10-Hydroxycarbazepine	[68–72]
Phenobarbital	15–30	55	70–140	10–40	43–172		[26,50,54,73–75]
Phenytoin	6–21	90	30–100‡‡	10–20	40–79		[1,50,54,56,73,76]
Pregabalin	1–2	0	5–7	NE§§	NE§§		Not validated
Primidone	2–5	10	7–22	5–10¶¶	23–46	Phenobarbital	[49,50,56,73]
Retigabine	1–2	80	8–10	NE§§	NE§§		Not validated
Rufinamide	1–2	35	6–10	30–40	126–168		[77]
Stiripentol	1–3	99	4.5–13‡‡	4–22	17–94		Not validated
Tiagabine	1–2	96	5–9	0.02–0.2	0.05–0.53		Not validated
Topiramate	4–7	15	20–30	5–20	15–59		[78]
Valproic acid	2–4	90	12–16	50–100	346–693		Salivary and serum valproic acid concentrations do not correlate.
Vigabatrin	1–2	0	5–8	0.8–36	6–279		[79]
Zonisamide	9–12	40	50–70	10–40	47–188		[80]

*For clarity, values can be rounded up or down by the laboratory.
†Refers to patients on chronic therapy after autoinduction is completed—values are much greater after a single dose.
‡There are clinical settings where monitoring of carbamazepine-epoxide, in addition to carbamazepine, is warranted, particularly when a comedication occurs with an inhibitor of carbamazepine–epoxide metabolism.
§Includes time to steady state for active metabolite *N*-desmethyl clobazam.
¶Refers to values for active metabolite *N*-desmethyl-clobazam.
#All values refer to the active metabolite eslicarbazepine.
**The reference range is that quoted for the active metabolite of oxcarbazepine, namely, 10-hydroxycarbazepine because the 2 molecules are identical.
††All values refer to the active metabolite 10-hydroxycarbazepine.
‡‡Elimination is saturable so that half-live increases with increasing plasma concentration.
§§Not established.
¶¶During treatment with primidone both primidone and the pharmacologically active metabolite phenobarbital should be monitored.

subject to many drug–drug pharmacokinetic interactions because its metabolism can be readily induced or inhibited—consequently there are large differences between individuals in the dose to serum concentration relationship. The current reference range for clobazam in serum is 0.03–0.3 mg/L (0.1–1.0 μmole/L), whereas that of *N*-desmethyl clobazam is 0.3–3.0 mg/L (1.0–10.5 μmole/L).[1]

Saliva TDM for Clobazam

There have been 2 studies investigating the distribution of clobazam and *N*-desmethyl clobazam into saliva and the relationship between salivary clobazam/*N*-desmethyl clobazam concentrations and serum total clobazam/*N*-desmethyl clobazam concentrations in children with epilepsy.[54,55] These showed that clobazam salivary concentration is similar to the total concentration in serum and that concentrations are significantly correlated ($r^2 = 0.90$). Also, *N*-desmethyl clobazam distributes into saliva such that the salivary concentration is similar to the total concentration in serum and that concentrations are significantly correlated ($r^2 = 0.93$). The excellent correlation between total serum clobazam/*N*-desmethyl clobazam and salivary concentrations ($r = 0.9$ and 0.93, respectively) indicates that saliva can be used as an alternative matrix for clobazam and *N*-desmethyl clobazam TDM.

Clonazepam

Clinical Indications

Clonazepam is licensed for the treatment of a variety of seizure types including absence, akinetic, atonic, and myoclonic seizures. Also, it is licensed for use in patients with

Lennox–Gastaut syndrome and in the management of status epilepticus. Clonazepam is available in a variety of formulations including tablets, a disintegrating wafer and a liquid formulation for intravenous administration.

Pharmacokinetic Characteristics

Clonazepam is rapidly absorbed after oral ingestion with a T_{max} of 1–4 hours and bioavailability of >80%. It exhibits linear pharmacokinetics and protein binding is 85%. Clonazepam is extensively metabolized in the liver, primarily by CYP3A4, to 7-aminoclonazepam, which in turn is metabolized by acetylation, via *N*-acetyl-transferase, to form 7-acetamidoclonazepam. The serum elimination half-life of clonazepam in adults is 17–56 hours so that interindividual clearance is extremely variable.[97–99] Clonazepam is subject to some drug–drug pharmacokinetic interactions consequent to the fact that its metabolism is readily induced or inhibited—therefore, large differences exist between individuals in the dose to serum concentration relationship. The current reference range for clonazepam in serum is 0.02–0.07 mg/L (0.06–0.22 μmole/L).[1]

Saliva TDM for Clonazepam

It is not known whether clonazepam is secreted into saliva and if it is whether the concentrations reflect those in serum. Analysis of saliva samples spiked with clonazepam and stored overnight at room temperature resulted in concentrations that were 76% lower compared with spiked saliva samples that were analyzed immediately.[100] These data suggest that clonazepam is unstable in saliva. Interestingly, clonazepam spiked into water was stable.[100]

Eslicarbazepine Acetate

Clinical Indications

Eslicarbazepine acetate is licensed for the adjunctive treatment of partial onset seizures with or without secondary generalization in patients with epilepsy aged 16 years and older. The drug is available as formulations of tablet and a suspension.

Pharmacokinetic Characteristics

Eslicarbazepine acetate is a prodrug, and after oral absorption, the acetate group is rapidly and extensively metabolized by hydrolytic first pass metabolism with esterases to eslicarbazepine (*S*-licarbazepine), the *S*-enantiomer of the pharmacologically active 10-hydroxycarbazepine metabolite of oxcarbazepine (also known as the monohydroxy derivative). After oral ingestion, eslicarbazepine acetate is rapidly absorbed with a T_{max} for eslicarbazepine of 2–3 hours and bioavailability of >90%.[101] Eslicarbazepine pharmacokinetics is linear and protein binding is 30%. In addition to eslicarbazepine, small amounts of 2 other pharmacologically active metabolites are formed from eslicarbazepine acetate (R-licarbazepine and oxcarbazepine), but these represent only approximately 6% of metabolites. Eslicarbazepine and its glucuronides together with minor quantities of R-licarbazepine, oxcarbazepine, eslicarbazepine acetate, and their respective glucuronides are excreted in urine. Eslicarbazepine glucuronic acid conjugation is primarily catalyzed by UGT1A4, UGT1A9, UGT2B4, UGT2B7, and UGT2B17.[102]

The serum half-life of eslicarbazepine in adults is 13–20 hours, and it is subject to very few drug–drug pharmacokinetic interactions.[103] The current reference range for eslicarbazepine in serum is 3–35 mg/L (12–139 μmole/L), which is based on that for racemic 10-hydroxycarbazepine derived from oxcarbazepine.[1]

Saliva TDM for Eslicarbazepine Acetate

It is not known whether eslicarbazepine acetate is secreted into saliva or whether salivary concentrations are similar or reflect those in serum. However, because the pharmacologically active metabolite, eslicarbazepine, is the same molecule as the pharmacologically active metabolite of oxcarbazepine, 10-hydroxycarbazepine, it can be expected that its transfer into saliva will be similar to that described for 10-hydroxycarbazepine in "Oxcarbazepine."

Ethosuximide

Clinical Indications

Ethosuximide is licensed for monotherapy treatment of absence seizures in patients of all ages and is available as formulations of capsule and a syrup.

Pharmacokinetic Characteristics

Ethosuximide is rapidly absorbed after oral ingestion with a T_{max} of 1–4 hours and its bioavailability is >90%. Its pharmacokinetics is linear and it is not protein bound.[104,105] Ethosuximide is extensively metabolized in the liver, primarily by CYP3A and to a lesser extent by CYP2E and CYP2B/C, to form isomers of 2-(1-hydroxymethyl)-2-methylsuccinamide of which 40% are excreted as glucuronide conjugates. The serum half-life of ethosuximide in adults is 40–60 hours with large interindividual differences in serum clearance. Furthermore, ethosuximide is subject to a number of drug–drug pharmacokinetic interactions because its metabolism can be both induced and inhibited with consequent large differences between individuals in the dose to serum concentration relationship. The current reference range for ethosuximide in serum is 40–100 mg/L (283–708 μmole/L).[1]

Saliva TDM for Ethosuximide

There have been several studies investigating the distribution of ethosuximide into saliva and the relationship between saliva ethosuximide concentrations and total serum ethosuximide concentrations in patients with epilepsy.[21,49,50,56–58] Ethosuximide is not protein bound and the salivary concentration is similar to the total serum concentration with mean saliva/serum total ethosuximide concentration ratios ranging 0.95–1.04. Indeed, saliva and serum total ethosuximide concentrations are significantly correlated ($r^2 = 0.99$), and thus, saliva can be used as an alternative matrix for ethosuximide TDM.

Felbamate

Clinical Indications

Because felbamate is associated with an increased risk of aplastic anemia and hepatotoxicity, its use is restricted such that it is approved for use only in patients who respond inadequately to alternative treatments and particularly in patients with

partial seizures or Lennox–Gastaut syndrome. Felbamate is available as formulations of tablet and a suspension.

Pharmacokinetic Characteristics

Felbamate is rapidly absorbed after oral ingestion with a T_{max} of 2–6 hours and a bioavailability of >90%. It has linear pharmacokinetics and protein binding is 25%. About 50% of an administered dose is metabolized in the liver, primarily by CYP3A4 and CYP2E1, to form 2 hydroxylated metabolites (*p*-hydroxy and 2-hydroxy felbamate). In addition one of the carbamate groups is hydrolyzed to an alcohol that is further biotransformed to an acid; also a number of as yet unidentified polar metabolites are produced, some of which are glucuronides.[106,107] The development of hepatotoxicity and aplastic anemia in a few patients treated with felbamate is due to the formation of a reactive atropaldehyde metabolite, which can accumulate in some patients and cause toxicity.[106] The serum half-life of felbamate in adults is 16–22 hours. Felbamate is subject to drug–drug pharmacokinetic interactions consequent to the fact that its metabolism is both readily induced and inhibited; also felbamate itself acts as an inhibitor of hepatic metabolism.[108–110] Consequently, there are large differences between individuals in the dose to serum concentration relationship. The current reference range for felbamate in serum is 30–60 mg/L (126–252 μmole/L).[1]

Saliva TDM for Felbamate

It is not known whether felbamate is secreted into saliva and if it is whether concentrations are similar to or reflect those in serum.

Gabapentin

Clinical Indications

Gabapentin is licensed for the monotherapy treatment of partial seizures with or without secondary generalization in adults and children aged 12 years and above, and as an adjunctive treatment in adults and children aged 6 years and above. The drug is also licensed for the treatment of peripheral neuropathic pain. Gabapentin is available as formulations of tablets and capsules.

Pharmacokinetic Characteristics

Gabapentin is rapidly absorbed after oral ingestion with a T_{max} of 2–3 hours. Bioavailability is 60% and is dose dependent with bioavailability decreasing at higher doses. Its pharmacokinetics is nonlinear consequent to its saturable absorption from the proximal small bowel primarily by the L-amino acid transport system.[111] Gabapentin is not protein bound and not metabolized, being cleared entirely by renal excretion with a serum elimination half-life in adults of 5–9 hours.[112] Nevertheless, although gabapentin is not subject to drug–drug pharmacokinetic interactions,[103] the disposition can be extremely variable because of wide interindividual differences is absorption.[113] The fact that gabapentin is associated with nonlinear pharmacokinetics is a major reason why gabapentin monitoring is particularly valuable for patient management and the current reference range for gabapentin in serum is 2–20 mg/L (12–117 μmole/L).[1]

Saliva TDM for Gabapentin

There have been 3 studies investigating the distribution of gabapentin in saliva—1 in healthy volunteers[59] and 2 in patients with epilepsy.[60,114] Gabapentin distributes into saliva; however, mean concentrations are 2.4–10% of those observed in serum.[59,60] Nevertheless, there is significant correlation (r^2 > 0.7) between saliva and serum total gabapentin concentrations[114]; furthermore, salivary gabapentin concentration and dose are significantly correlated (r^2 = 0.77–0.95).[59,60] Thus, saliva may be a useful alternative matrix for gabapentin TDM.

Lacosamide

Clinical Indications

Lacosamide is licensed for the adjunctive treatment of partial onset seizures with or without secondary generalization in patients with epilepsy aged 16 years and older. Lacosamide is available as formulations of tablets, a solution, and a syrup.

Pharmacokinetic Characteristics

Lacosamide is rapidly absorbed after oral ingestion with a T_{max} of 1–2 hours, and its bioavailability is 100%.[115] Its pharmacokinetics is linear and protein binding is controversial with Greenaway et al[61] reporting 90% and the Summary of Product Characteristic[116] stating that serum lacosamide protein binding is <15%. About 60% of a dose of lacosamide is hepatically metabolized, by demethylation via CYP2C19, to form *O*-desmethyl lacosamide.[102] The serum elimination half-life of lacosamide in adults is 13 hours[115] and to date no drug–drug pharmacokinetic interactions have been identified.[103] The current reference range for lacosamide in serum is 10–20 mg/L (40–80 μmole/L).

Saliva TDM for Lacosamide

There has been one study investigating the distribution of lacosamide into saliva and the relationship between saliva lacosamide concentration and both serum total and free concentrations in adults with epilepsy.[61] Lacosamide distributes into saliva such that the salivary concentration is similar to the non–protein bound concentration in serum; mean saliva/serum-free lacosamide concentration ratios ranged 0.77–0.96. Furthermore, saliva lacosamide concentrations are significantly correlated with both serum total lacosamide (r^2 = 0.84) and serum-free lacosamide (r^2 = 0.83) concentrations. Thus, saliva should be a useful alternative matrix for lacosamide TDM.

Lamotrigine

Clinical Indications

Lamotrigine is licensed for the monotherapy treatment of partial seizures and primary and secondarily generalized tonic–clonic seizures in adults and children over 12 years of age; as adjunctive treatment in adults and children over 2 years of age; as adjunctive treatment of seizures associated with the Lennox–Gastaut syndrome in adults and children over 2 years of age. Lamotrigine is also licensed for the treatment of bipolar I disorder. It is available as formulations of tablets and dispersible chewable tablets.

Pharmacokinetic Characteristics

Lamotrigine is rapidly absorbed after oral ingestion with a T_{max} of 1–3 hours and its bioavailability is >95%.[117] Its pharmacokinetics is linear and protein binding is 55%. Lamotrigine is extensively metabolized in the liver, primarily by glucuronidation via UGT1A4, to form N-2 and N-5 glucuronides.[117] The serum elimination half-life of lamotrigine in adults is 15–35 hours so that interindividual clearance is extremely variable[118]; furthermore, it is subject to many drug–drug pharmacokinetic interactions consequent to the fact that its metabolism can be both induced and inhibited; consequently, there are large differences between individuals in the dose to serum concentration relationship.[103] The current reference range for lamotrigine in serum is 2.5–15 mg/L (10–59 μmole/L).[1]

Saliva TDM for Lamotrigine

Lamotrigine is reported to be about 55% bound to serum proteins in patients receiving 150–300 mg/d in conjunction with other medication and the saliva/serum lamotrigine ratio is reported to be 0.46 in healthy subjects receiving a single dose and 0.56 in patients receiving adjunctive therapy.[119,120] The excellent correlation between serum and saliva concentrations of lamotrigine ($r = 0.95$) in these early studies suggested that saliva could potentially be used to monitor the systemic concentrations of lamotrigine.

A subsequent study examined the interindividual correlation between lamotrigine concentrations in saliva and serum together with the relationship between saliva concentration and the non–protein bound lamotrigine concentration in serum.[62] The authors compared both stimulated and unstimulated saliva from the same patients and demonstrated a good correlation between lamotrigine serum concentration in both collection modes ($r^2 = 0.85$, unstimulated and $r^2 = 0.94$, stimulated). Furthermore, the study demonstrated a good correlation between total lamotrigine concentration in serum and the free concentration as determined by ultrafiltration ($r^2 = 0.95$) and equilibrium dialysis ($r^2 = 0.93$). Lamotrigine concentration in stimulated saliva was also significantly correlated with the free concentration and calculation of lamotrigine protein binding using the 3 alternative procedures gave the following results (mean ± SD); 51.8% ± 13.03% (stimulated), 68.05% ± 7.59% (ultrafiltration), and 58.72% ± 7.68% (equilibrium dialysis). The differences in calculated binding between the 3 methods were significant.[62]

Ryan et al[63] studied the relationship between serum and salivary concentrations of lamotrigine in both pediatric and adult epilepsy populations and reported a good correlation between the two ($r^2 = 0.81$–0.84) and with the saliva/serum lamotrigine concentration ratios ranging 0.40–1.19 (mean ± SD = 0.64 ± 0.18). The authors concluded that although a good correlation existed for the population at large between salivary and serum concentrations for lamotrigine, there is wide interpatient variability in the saliva/serum ratio. The data suggest that salivary monitoring may play a role in the monitoring of lamotrigine for adult and pediatric patients.

In another study, lamotrigine concentrations were measured in both stimulated and unstimulated saliva alongside matching serum samples from 7 adult volunteers over a 32-hour period after a single 50-mg dose of the drug, also in samples from 20 children and adolescents during the course of routine AED therapy.[64] In specimens collected ≥2 hours after ingestion, there was a close correlation in each individual between the concentrations in stimulated and unstimulated saliva, which were similar. The saliva/serum lamotrigine concentration ratio gave a mean value of 0.49 at a serum lamotrigine concentration of 10 mg/L, and the authors concluded that with appropriate precautions regarding the timing of sample collection saliva measurements could provide a reasonable alternative to serum for TDM.[64]

A study in 14 healthy volunteers comparing saliva and serum lamotrigine concentrations over 96 hours after ingestion of a single oral dose of lamotrigine reported significant correlation between saliva and serum ($r^2 = 0.677$).[121] Furthermore, the mean saliva/serum lamotrigine concentration ratio was 0.425 ± 0.153, and the calculated protein binding from the concentration in saliva was 57.5 ± 15.1% (mean ± SD); thus, saliva concentrations reflect the free concentrations in serum. More recently, Mallayasamy et al[122] reported a correlation between salivary and serum lamotrigine concentrations of 0.683.

In summary, for lamotrigine, there are many factors that make TDM clinically useful, and several studies have found good correlation between salivary concentrations and both total and free non–protein bound serum concentrations; thus, lamotrigine TDM in saliva is a viable alternative to that of serum.

Levetiracetam

Clinical Indications

Levetiracetam is licensed for the monotherapy treatment of partial seizures with or without secondary generalization in patients aged 16 years and older and as adjunctive treatment in adults and children from 4 years of age. The drug is also licensed for the adjunctive treatment of primary generalized tonic–clonic seizures associated with idiopathic generalized epilepsy and myoclonic seizures associated with juvenile myoclonic epilepsy in adults and adolescents from 12 years of age. Levetiracetam is available in a variety of formulations including tablets, an oral solution, a solution for intravenous injection, and an extended release tablet formulation.

Pharmacokinetic Characteristics

Levetiracetam is rapidly absorbed after oral ingestion with a T_{max} of 1–2 hours and a bioavailability of >95%. Its pharmacokinetics is linear, and it is not protein bound.[123] Approximately 30% of a dose of levetiracetam undergoes metabolism by a cytosolic amidase enzyme to produce a carboxylic acid metabolite (2-pyrrolidone-*N*-butyric acid), which is excreted unchanged via the kidneys.[124,125] Levetiracetam metabolism to the carboxylic acid is independent of the hepatic CYP system and occurs by means of a type-B esterase located in whole blood.[126] However, the drug also undergoes a small amount of hepatic metabolism to form 2 ring-hydroxylated metabolites. The serum elimination half-life of levetiracetam in adults is 6–8 hours, and it is subject to minimal drug–drug pharmacokinetic interactions.[103] The current reference range for levetiracetam in serum is 12–46 mg/L (70–270 μmole/L).[1]

Saliva TDM for Levetiracetam

There have been several studies investigating the distribution of levetiracetam into saliva and the relationship between saliva levetiracetam and serum concentrations in adults and children with epilepsy and also in healthy volunteers.[65–67]

Levetiracetam distributes into saliva. However, there is some controversy regarding whether or not saliva and serum levetiracetam concentrations are the same. Grim et al[65] report that salivary concentrations are approximately 40% of that observed in serum, with mean saliva/serum levetiracetam concentration ratios ranging 0.36–0.41. In contrast, Lins et al[66] and Mecarelli et al[67] report that the concentration of levetiracetam in saliva is similar to serum with the mean saliva/serum levetiracetam concentration ratio being 1.0 and 1.1, respectively. Interestingly, a ratio of 1.55 was observed after ingestion of an oral solution in healthy volunteers.[66] Nevertheless, saliva and serum levetiracetam concentrations are significantly correlated (r^2 = 0.86–0.91) although lemon juice stimulation reduces the correlation from r^2 = 0.91 to r^2 = 0.87. Overall, the data suggest that saliva may be used as an alternative matrix for levetiracetam TDM.

Oxcarbazepine

Clinical Indications

Oxcarbazepine is licensed for the monotherapy or adjunctive treatment of partial seizures with or without secondary generalization in patients aged 6 years or more. It is available as formulations of tablets and an oral suspension.

Pharmacokinetic Characteristics

Oxcarbazepine is a prodrug and is rapidly metabolized, by cytosolic arylketone reductase, to a pharmacologically active metabolite 10-hydroxycarbazepine (also known as licarbazepine or monohydroxy metabolite). This metabolite accumulates in serum and is responsible for most of the drug effects. The conversion of oxcarbazepine to 10-hydroxycarbazepine is stereoselective and concentrations of the *S*-enantiomer are somewhat higher than those of the *R*-enantiomer.[127,128] After oral ingestion, oxcarbazepine is rapidly absorbed with a T_{max} of 3–6 hours and a bioavailability of 100%. Oxcarbazepine pharmacokinetics is linear and protein binding is 60%, whereas 10-hydroxycarbazepine protein binding is 40%.[129] 10-Hydroxycarbazepine is subsequently metabolized by conjugation with glucuronic acid, and the conjugates together with some 10-hydroxycarbazepine are excreted in urine. The serum elimination half-life of 10-hydroxycarbazepine in adults is 8–15 hours, and it is subject to many drug–drug pharmacokinetic interactions because its metabolism is both readily inhibited and induced. Oxcarbazepine is also itself a weak inducer of hepatic metabolism[130,131]; consequently, there are large differences between individuals in the dose to serum concentration relationship. The current reference range for 10-hydroxycarbazepine in serum is 3–35 mg/L (12–139 μmole/L).[1]

Saliva TDM for 10-Hydroxycarbazepine

There have been several studies investigating the distribution of 10-hydroxycarbazepine into saliva and the relationship between salivary concentrations and serum total/free 10-hydroxycarbazepine concentrations in healthy volunteers and in adults and children with epilepsy.[68–72] 10-Hydroxycarbazepine distributes into saliva; however, the extent depends on whether resting saliva or stimulated saliva is collected. Thiesohn and Heimann[68] in a study of 3 healthy volunteers observed that in resting saliva the saliva/serum ratio ranged 0.3–1.7 (median 1.0). Kristensen et al[69] in a study of 7 healthy volunteers reported that in stimulated saliva (paraffin wax) the mean saliva/serum ratio was 0.53. The study of Klitgaard and Kristensen[70] involving 17 patients with epilepsy reported that in resting saliva the mean saliva/serum ratio was 1.01, whereas in stimulated saliva (paraffin wax), the value was 0.41. Cardot et al[71] in a study of 10 patients with epilepsy reported that in stimulated saliva (citric acid) the mean saliva/serum ratio was 0.19. These data indicate that with greater saliva stimulation the saliva/serum ratio decreases such that saliva 10-hydroxycarbazepine concentrations approached the free 10-hydroxycarbazepine concentrations in serum. This may be due to the relatively low lipid solubility of 10-hydroxycarbazepine; a characteristic that is not associated with carbamazepine. Unfortunately, increasing salivary flow has an extremely variable effect on saliva 10-hydroxycarbazepine concentration, which results in the saliva and serum 10-hydroxycarbazepine concentrations not being significantly correlated. However, when unstimulated saliva is collected, there is a significant correlation between saliva and serum total 10-hydroxycarbazepine concentrations (r^2 = 0.91–0.98).[68–70] Indeed, a recent study by Miles et al[72] of 28 children and adult patients with epilepsy reported on unstimulated saliva whereby the mean saliva/serum ratio was 0.96 and saliva 10-hydroxycarbazepine concentrations were significantly correlated with serum total 10-hydroxycarbazepine concentrations (r^2 = 0.94). Thus unstimulated saliva may be a useful alternative matrix for 10-hydroxycarbazepine TDM.

Phenobarbital

Clinical Indications

Phenobarbital is licensed for the monotherapy or adjunctive treatment of all forms of epilepsy, except absence seizures, in patients of any age. Phenobarbital is available in a variety of formulations including, tablets, a solution for intravenous injection, and an elixir formulation.

Pharmacokinetic Characteristics

Phenobarbital is rapidly absorbed after oral ingestion with a T_{max} of 2–4 hours and a bioavailability of >90.[132,133] Its pharmacokinetics is linear, and protein binding is 55%. Phenobarbital is extensively metabolized in the liver, primarily by CYP2C9 and to a lesser extent by CYP2C19 and CYP2E1, to form 2 major metabolites, *p*-hydroxyphenobarbital and a 9-D-glucopyranosyl phenobarbital isomer. The serum half-life of phenobarbital in adults is 70–140 hours so that interindividual clearance is extremely variable[132,133] Phenobarbital is subject to many drug–drug pharmacokinetic interactions consequent to the fact that it is a potent inducer of hepatic metabolism and also its own metabolism can be induced or inhibited. Consequently, there are large differences between individuals in the dose to serum concentration relationship. The current reference range for phenobarbital in serum is 10–40 mg/L (43–172 μmole/L).[1]

Saliva TDM for Phenobarbital

Using saliva as an alternative matrix for TDM of phenobarbital is somewhat controversial because there is no clear consensus regarding whether the concentration in saliva directly reflects the concentrations of the drug in serum. A number of studies in both adults and children with epilepsy have demonstrated that phenobarbital distributes into saliva with saliva/total serum phenobarbital concentration ratios ranging 0.2–0.52, whereas the mean saliva/serum-free phenobarbital concentration ratios ranged 0.63–0.68. Indeed, saliva phenobarbital concentrations and both serum total phenobarbital (r^2 = 0.65–0.98) and serum-free phenobarbital (r^2 = 0.64–0.99) concentrations are significantly correlated.[26,49,50,53,54,56,57,73–75,134–137]

Two studies have demonstrated that the distribution of phenobarbital into saliva depends on salivary pH[56,136]; however, other studies have not found an effect of pH.[75,137] The study by McAuliffe et al[56] determined phenobarbital in saliva and serum obtained simultaneously from 115 patients, and a method to correct for the effect of salivary pH on drug concentration of saliva was developed. Salivary phenobarbital concentration was found to be equivalent to the free phenobarbital concentration in serum and to correlate significantly with the total serum concentration. Expressed as percentage of total serum drug, the salivary (*S*) and serum-free (*P*) concentrations were: phenobarbital, *S* 43.1 ± 5.2%, *P* = 40.8 ± 7.9% (r = 0.91). On balance, it seems that salivary concentrations of phenobarbital correlate with the simultaneous serum water concentrations, after correcting for the effects of pH differences between saliva and serum.[138]

Despite the contradictory results, when equations incorporate the relative ratio of phenobarbital pK_a to the salivary pH, there is an excellent correlation between salivary and free non–protein bound serum phenobarbital concentrations. Thus, saliva can be regarded as a useful alternative matrix for phenobarbital TDM.

Phenytoin

Clinical Indications

Phenytoin is licensed for both monotherapy and adjunctive therapy of clonic–clonic seizures and focal seizures in patients of any age. It is also approved for the treatment of seizures occurring during or after neurosurgery and/or severe head injury. In addition, it is licensed for intravenous administration in the management of established status epilepticus and for monotherapy use in the treatment of trigeminal neuralgia. Phenytoin is available in a variety of formulations including, capsules, chewable tablets, an oral suspension and a parenteral solution formulation. More recently, fosphenytoin has been licensed, which is a water-soluble phenytoin phosphate prodrug that is rapidly dephosphorylated on administration. It is formulated for intravenous administration to control of status epilepticus and the prevention/treatment of seizures occurring in connection with neurosurgery and/or head trauma. It can also be substituted for oral phenytoin if oral administration is not possible and/or contraindicated. Fosphenytoin is formulated as a solution for infusion or injection.

Pharmacokinetic Characteristics

The rate of absorption of phenytoin after oral ingestion is variable and formulation dependent with a T_{max} of 1–12 hours.[139] Bioavailability is similarly formulation dependent but is >80%. Its pharmacokinetics is nonlinear due to saturable metabolism, which results in Michaelis–Menten kinetics within the range of serum concentrations that are generally associated with its beneficial therapeutic effects.[140] The protein binding of phenytoin is 90%. Phenytoin is extensively metabolized in the liver, primarily by CYP2C9 and CYP2C19, to form 2 major metabolites 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (which undergoes partial conversion to glucuronides before renal excretion) and a dihydrodiol derivative.[141] The serum elimination half-life of phenytoin in adults is 30–100 hours so that interindividual clearance is extremely variable. Phenytoin is subject to many drug–drug pharmacokinetic interactions consequent to the fact that it is a potent inducer of hepatic metabolism; also its metabolism can be both induced and inhibited—consequently, there are large differences between individuals in the dose to serum concentration relationship. The fact that phenytoin is associated with nonlinear pharmacokinetics is one of the major reasons why monitoring the drug is so useful. The current reference range for phenytoin in serum is 10–20 mg/L (40–79 μmole/L).[1]

Saliva TDM for Phenytoin

There have been many studies investigating the distribution of phenytoin into saliva and the relationship between saliva phenytoin concentration and both serum total and free phenytoin concentrations in both adults and children with epilepsy.[26,49,53,54,56,57,73,76,90,95,134,135,137,142–146] Phenytoin distributes into saliva such that the salivary concentration is similar to the free non–protein bound concentration in serum. Mean saliva/serum total phenytoin concentration ratios ranged 0.09–0.13, whereas the mean saliva/serum-free phenytoin concentration ratios ranged 0.99–1.06. Indeed, saliva phenytoin concentrations and both serum total phenytoin (r^2 = 0.85–0.99) and serum-free phenytoin (r^2 = 0.96–0.99) concentrations are significantly correlated. There is a suggestion that the extent of phenytoin distribution in saliva depends on whether resting saliva, stimulated saliva, or reduced flow saliva is collected. For the latter 2 situations, phenytoin concentrations in saliva are decreased and increased, respectively.[147] Thus, unstimulated saliva should be used, and this provides an extremely useful alternative matrix for phenytoin TDM.

Pregabalin

Clinical Indications

Pregabalin is licensed as adjunctive treatment of partial seizures with or without secondary generalization in adults. It is also licensed for the treatment of peripheral and central pain and for generalized anxiety disorders. Pregabalin is available as formulations of capsules only.

Pharmacokinetic Characteristics

Pregabalin is rapidly absorbed after oral ingestion with a T_{max} of 1–2 hours and bioavailability of >90%.[148] Its

pharmacokinetics is linear, and it is not protein bound. Pregabalin is not metabolized being cleared entirely by renal excretion with a serum elimination half-life of 5–7 hours in adults.[148] Pregabalin is not subject to drug–drug pharmacokinetic interactions.[103] The precise role for TDM of pregabalin has not yet been established, although there may be a requirement in patients with renal impairment, to ascertain compliance, where malabsorption is suspected and in cases of suspected overdose. Very little information is available regarding therapeutic serum concentrations of pregabalin; however, one report states that in samples collected at random times relative to dose from patients maintained on 600 mg/d, serum pregabalin concentrations ranged from 0.9 to 14.2 mg/L. There are reports of significant toxicity in a case of self-poisoning with pregabalin alone[149]; also, a case of toxicity associated with therapeutic use in a patient with renal failure although the peak drug concentration (predialysis) in this case was only 13 mg/L.

Saliva TDM for Pregabalin

It is not known whether pregabalin is secreted into saliva and if it is whether concentrations are similar to or reflect those in serum.

Primidone

Clinical Indications

Primidone is licensed for the monotherapy or polytherapy treatment of generalized tonic–clonic seizures, psychomotor and focal seizures in adults and children and for the management of Jacksonian seizures, myoclonic jerks, and akinetic attacks. It is also licensed for the treatment of essential tremor. Primidone is available as tablet formulations and a suspension.

Pharmacokinetic Characteristics

Primidone is rapidly absorbed after oral ingestion with a T_{max} of 2–4 hours and bioavailability of >90%.[150] Its pharmacokinetics is linear and protein binding is 10%. Primidone is extensively metabolized in the liver to form 2 major pharmacologically active metabolites, phenobarbital and phenyl-ethyl-malonamide. The serum elimination half-life of primidone in adults is 7–22 hours so that interindividual clearance is variable.[150] Primidone is subject to many drug–drug pharmacokinetic interactions consequent to the fact that it is a potent inducer of hepatic metabolism (via phenobarbital). Also, its metabolism can be induced or inhibited; consequently, there are large differences between individuals in the dose to serum concentration relationship. During treatment with primidone, it is common practice to monitor phenobarbital because the adverse effects from a high phenobarbital concentration is more likely to limit a primidone dosage increase. The current reference range for primidone in serum is 5–10 mg/L (23–46 μmole/L).[1]

Saliva TDM for Primidone

There have been several studies that investigate the distribution of primidone in saliva and the relationship between saliva primidone and serum total primidone concentrations in both adults and children with epilepsy.[26,49,50,56,57,73,151] The mean saliva/serum total primidone concentration ratios ranged 0.07–1.15. Furthermore, saliva and total serum primidone concentrations are significantly correlated (r^2 = 0.71–0.97). Salivary primidone concentrations seem to be flow dependent with resting saliva concentrations being approximately 38% lower than in flow-stimulated saliva.[152] Thus, provided that sample collection is standardized saliva may be used as an alternative matrix for primidone TDM.

Retigabine

Clinical Indications

Retigabine is indicated as adjunctive treatment of partial onset seizures with or without secondary generalization in adults aged 18 years and above with epilepsy. Retigabine is available as formulations of tablet only.

Pharmacokinetic Characteristics

Retigabine is rapidly absorbed after oral ingestion with a T_{max} of 0.6–1.5 hours and bioavailability of about 60%.[152] Its pharmacokinetics is linear and protein binding is 80%.[153] Approximately 20%–30% of the administered dose of retigabine is eliminated renally unchanged with the remaining drug being biotransformed to produce the *N*-acetyl metabolite (which has a weak pharmacological action) and N-glucuronide conjugates of both parent drug and *N*-acetylretigabine accumulates in plasma reaching similar concentrations to the parent drug and while the *N*-glucuronide metabolites are pharmacologically inactive, they may contribute to the enterohepatic circulation of retigabine and *N*-acetylretigabine.[102] The serum elimination half-life of retigabine in adults is 8–10 hours, and it is subject to only a few drug–drug pharmacokinetic interactions.[103] The precise role for TDM of retigabine has not yet been established, although there may be a requirement in patients to ascertain compliance, where malabsorption is suspected and in cases of suspected toxicity. There are no data relating plasma retigabine levels with that of seizure suppression or adverse effects.

Saliva TDM for Retigabine

It is not known whether retigabine is secreted into saliva and if so whether concentrations are similar to or reflect those in serum.

Rufinamide

Clinical Indications

Rufinamide is licensed for the adjunctive treatment of seizures associated with the Lennox–Gastaut syndrome in patients 4 years and older. Rufinamide is available as formulations of tablet only.

Pharmacokinetic Characteristics

Rufinamide is rapidly absorbed after oral ingestion with a T_{max} of 4–6 hours and bioavailability decreases with increasing dose. Consequently, its pharmacokinetics is linear up to only 1600 mg/d. Protein binding is 35%.[154,155] Because food coingestion enhances C_{max} values by 50% and increases area under the concentration versus time curve values by 33%, probably by improving the solubility of rufinamide, patients should be advised to take their rufinamide dose each

time in the same temporal relation to their meals to maintain steady-state concentrations from one dose to the next.[156] Rufinamide is extensively metabolized in the liver, primarily by an amidase hydrolysis (which is not CYP dependent) that converts the carboxamide function to the corresponding carboxylic acid, which is not pharmacologically active.[154,157] The acid subsequently undergoes glucuronidation before renal excretion. The serum elimination half-life of rufinamide in adults is 6–10 hours.[158] Rufinamide is subject to some drug–drug pharmacokinetic interactions consequent to the fact that its metabolism can be both induced and inhibited[103]—consequently, there are large differences between individuals in the dose to serum concentration relationship. Furthermore, because rufinamide is associated with nonlinear pharmacokinetics, there is an excellent rational for rufinamide TDM. The current information indicates that serum concentrations in the range of 30–40 mg/L (126–168 μmole/L) are required for seizure control in patients with Lennox–Gastaut syndrome; however, lower concentrations may prove to be effective in other seizure types.[154,155]

Saliva TDM for Rufinamide

Rufinamide distributes into saliva and preliminary measurements in a single patient of saliva and serum samples at steady state after 3 different rufinamide doses indicate that the mean saliva to serum concentration ratio was 0.66.[77] Because the serum protein binding of rufinamide is 35%, these data indicate that salivary rufinamide concentrations reflect the non–protein bound drug concentrations in serum.[154] However, a larger population of patients will require testing to confirm this preliminary finding and also a direct comparison between the concentration in saliva and the unbound concentration in serum will need to be performed. Nevertheless, it seems that TDM of rufinamide in saliva is likely to be a useful alternative to serum, particularly if salivary concentrations of rufinamide reflect the free, pharmacologically active concentration in serum.

Stiripentol

Clinical Indications

Stiripentol is licensed for the adjunctive treatment of seizures in children with severe myoclonic epilepsy in infancy (Dravet syndrome). Stiripentol is available as formulations of capsule- and sachet-containing granules.

Pharmacokinetic Characteristics

Stiripentol is rapidly absorbed after oral ingestion with a T_{max} of 0.5–2.0 hours; however, its bioavailability has yet to be determined.[159] Stiripentol pharmacokinetics is nonlinear due to saturable metabolism, which results in Michaelis–Menten kinetics within the range of serum concentrations that are generally associated with beneficial therapeutic effects.[160] Protein binding is 99%, and the drug is subject to extensive first pass metabolism.[159] The serum elimination half-life of stiripentol in adults is 4.5–13 hours, and its metabolism is complex with 13 different metabolites having been identified.[161,162] The principal hepatic enzymes involved are CYP1A2, CYP2C19, and CYP3A4 and interindividual clearances of stiripentol are extremely variable. Stiripentol is subject to many drug–drug pharmacokinetic interactions consequent to the fact that it is a potent inhibitor of hepatic metabolism and also its own metabolism can be induced[103]; therefore, large differences occur between individuals in the dose to serum concentration relationship. The nonlinear pharmacokinetics of stiripentol is the primary reason why monitoring is useful. Although the reference range for stiripentol in serum is not well defined, concentrations of 4–22 mg/L (17–94 μmole/L) correlate with control of absence seizures in children[163] and in Dravet syndrome concentrations of 8–12 mg/L (34–51 μmole/L) are reported to be effective.[164]

Saliva TDM for Stiripentol

It is not known whether stiripentol is secreted into saliva and if it is whether concentrations are similar to or reflect those in serum. However, if the concentration reflects the free non–protein bound concentration it will present an analytical challenge because of the 99% binding of stiripentol.

Tiagabine

Clinical Indications

Tiagabine is licensed for the adjunctive treatment of partial seizures with or without secondary generalization in adults and children aged 12 years and above. Tiagabine is available as formulations of tablet only.

Pharmacokinetic Characteristics

Tiagabine is rapidly absorbed after oral ingestion with a T_{max} of 0.5–2 hours and bioavailability is >90%. Its pharmacokinetics is linear and protein binding is 96%. Tiagabine is extensively metabolized in the liver, primarily by CYP3A4, to form two 5-oxo-tiagabine isomers together with some additional minor metabolites.[165] The serum elimination half-life of tiagabine in adults is 5–9 hours. The drug is subject to drug–drug pharmacokinetic interactions consequent to the fact its metabolism can be readily induced[103]; therefore, large differences occur between individuals in the dose to serum concentration relationship.[166] The current reference range for tiagabine is 0.02–0.2 mg/L (0.05–0.53 μmole/L).[1] However, Uthman et al[167] showed in their patient group that the reduction in the frequency of complex partial seizures was related to serum tiagabine concentrations with the best seizure control occurring at trough concentrations above 0.04 mg/L (0.11 μmole/L), whereas concentrations of 0.4 mg/L (1.06 μmole/L) were associated with central nervous system toxicity.

Saliva TDM for Tiagabine

It is not known whether tiagabine is secreted into saliva and if it is whether concentrations are similar to or reflect those in serum. However, because serum concentrations are 1–2 orders of magnitude less than most other AEDs and with a protein binding of 96%, measurement of the free non–protein bound concentration will be analytically challenging.

Topiramate

Clinical Indications

Topiramate is licensed for the monotherapy treatment of generalized tonic–clonic seizures and partial seizures with

or without secondarily generalization in adults and children aged 6 years and above; also as adjunctive therapy for adults and children aged 2 years and above. It is also licensed for the adjunctive treatment of seizures associated with Lennox–Gastaut syndrome and for primary generalized tonic–clonic seizures. Topiramate is also licensed for the treatment of migraine and is available as formulations of tablet and a sprinkle capsule.

Pharmacokinetic Characteristics

Topiramate is rapidly absorbed after oral ingestion with a T_{max} of 2–4 hours and bioavailability is >80%. Its pharmacokinetics is linear and protein binding is 15%.[168] Approximately 50% of a dose of topiramate is excreted unchanged by the kidneys with the remainder being metabolized in the liver, primarily by yet to be identified CYP isoenzymes, to form several oxidative metabolites some of which are conjugated.[169,170] The serum elimination half-life of topiramate in adults is 20–30 hours and the drug is subject to many drug–drug pharmacokinetic interactions consequent to the fact that it is a weak inducer of hepatic metabolism; furthermore, its own metabolism can be enhanced by hepatic enzyme inducers and some drugs also inhibit topiramate metabolism.[103] Consequently, there are large differences between individuals in the dose to serum concentration relationship. The current reference range for topiramate in serum is 5–20 mg/L (15–59 μmole/L).[1]

Saliva TDM for Topiramate

There has been one study investigating the distribution of topiramate into saliva and the relationship between salivary and serum topiramate concentration (both total and free) in adults and children with epilepsy.[78] Topiramate distributes into saliva such that the salivary concentration is similar to the total concentration in serum with saliva/serum total concentration ratios ranging 0.63–1.13 (mean 0.90 ± 0.12). The concentrations of topiramate in saliva are significantly correlated ($r^2 = 0.97$) with the total in serum, which enables saliva to be used as an alternative matrix for topiramate TDM.

Valproate

Clinical Indications

Valproate is licensed for monotherapy or adjunctive treatment of any form of epilepsy in patients of any age. It is also licensed for the treatment of migraine and bipolar disorder. Valproate is available in a variety of formulations including, enteric-coated tablets, chewable tablets, capsules, sustained-release tablets and microspheres, modified release granules, a liquid oral solution, a syrup, and a solution for intravenous injection.

Pharmacokinetic Characteristics

Valproic acid is rapidly absorbed after oral ingestion; however, T_{max} values are formulation dependent and variable (1–7 hours). Bioavailability is generally >90% but is 8%–20% lower for extended release formulations. The serum protein binding of valproate is normally 90% but is concentration dependent and saturable, which causes the pharmacokinetics of the drug to be nonlinear.[171] Valproic acid is extensively metabolized in the liver and metabolism is complex in that it involves multiple metabolic pathways including O-glucuronidation, β-oxidation, ω-oxidation, hydroxylation, ketone formation, and desaturation. In excess of 25 metabolites have been identified but valproic acid glucuronide and 3-oxo-valproic acid are by far the most abundant. The serum elimination half-life of valproic acid in adults is 12–16 hours,[172] which reflects the interindividual variation in clearance and it is subject to many drug–drug pharmacokinetic interactions.[103] Valproic acid is a potent inhibitor of hepatic metabolism and furthermore its own metabolism can be both induced and inhibited; consequently, there are large differences between individuals in the dose to serum concentration relationship. The current reference range for total valproic acid in serum is 50–100 mg/L (346–693 μmole/L),[1] but the measurement of free non–protein bound valproic acid concentration is more clinically useful because of the large variability in protein binding.

Saliva TDM for Valproate

The physicochemical properties of valproic acid (ie, a weak acid with a pK_a of 4.9), and the pH gradient between serum and saliva result in only small quantities of valproic acid passing into saliva; furthermore, concentrations are erratic. Stimulation by citric acid did not increase the low saliva valproic acid concentrations, and the correlation between saliva and serum valproic acid concentrations (both total and free) was poor.[54,173] Because salivary valproic acid concentrations correlate poorly with total and free serum valproic acid concentrations,[54,95,173–176] saliva cannot be used as an alternative matrix for valproic acid TDM.

Vigabatrin

Clinical Indications

Vigabatrin is licensed for the adjunctive treatment of partial seizures with and without secondary generalization. It is also licensed for the monotherapy treatment of infantile spasms (West Syndrome). Vigabatrin is available as formulations of a tablet and a sachet (containing granules) only.

Pharmacokinetic Characteristics

Vigabatrin is rapidly absorbed after oral ingestion with a T_{max} of 1–2 hours and a bioavailability of 60%–80%. Its pharmacokinetics is linear, and it is not protein bound.[177] Vigabatrin is not metabolized being cleared entirely by renal excretion with a serum elimination half-life of 5–8 hours in adults.[178] Vigabatrin is not subject to drug–drug pharmacokinetic interactions.[103] It acts by elevating brain γ-amino butyric acid (GABA) concentrations. GABA is the principal inhibitory neurotransmitter in the brain, and vigabatrin acts through a mechanism whereby it selectively and irreversibly binds to GABA-transaminase (GABA-T) thus blocking the enzyme responsible for GABA metabolism and increasing GABA concentrations.[179] GABA concentrations remain elevated for some time after vigabatrin can be detected in serum because GABA metabolism depends on resynthesis of GABA-T.[180] Because of its mechanism of action, TDM of vigabatrin is generally considered not to be very helpful and a wide range of trough serum concentrations have been found in patients successfully treated with the drug. However, TDM may be useful in patients with renal impairment, to

ascertain compliance, where malabsorption is suspected and in cases of suspected overdose. The current reference range for vigabatrin in serum is 0.8–36 mg/L (6–279 μmole/L).[1]

Saliva TDM for Vigabatrin

After administration of a single oral dose of vigabatrin to 6 healthy volunteers, salivary vigabatrin concentration was approximately 10% of that in serum.[79] Thus, salivary vigabatrin concentrations do not reflect the free non–protein bound concentrations in serum. There are no data as to the correlation between salivary and serum vigabatrin concentrations, and therefore, it is uncertain at present as to whether saliva could be used as an alternative matrix for vigabatrin TDM.

Zonisamide

Clinical Indications

Zonisamide is licensed for the adjunctive treatment of partial seizures with and without secondary generalization in adults. Zonisamide is available only as formulations of a capsule.

Pharmacokinetic Characteristics

Zonisamide is rapidly absorbed after oral ingestion with a T_{max} of 2–5 hours and bioavailability of >90%. Its pharmacokinetics is linear, and protein binding is 40%; also, the drug has a high affinity, low capacity-binding site on erythrocytes.[181] Zonisamide is extensively metabolized in the liver, primarily by acetylation, to form *N*-acetyl zonisamide and reduction catalyzed by CYP3A4, to form 2-sulfamoylacetylphenol, which is subsequently glucuronidated.[182] The serum half-life of zonisamide in adults is 50–70 hours so that interindividual clearance is variable.[183–185] Zonisamide is subject to some drug–drug pharmacokinetic interactions consequent to the fact that its metabolism can be induced or inhibited[103]; consequently, there are large differences between individuals in the dose to serum concentration relationship. The current reference range for zonisamide in serum is 10–40 mg/L (47–188 μmole/L).[1]

Saliva TDM for Zonisamide

Zonisamide distributes into saliva, but limited information is available relating salivary concentrations to those in serum. The drug seems to be actively secreted into saliva because concentrations are reported to be higher than the free concentration in serum.[80] More recently, Jones et al[186] included saliva samples from patients prescribed zonisamide in a mailing stability study, and although they demonstrated that the drug was stable in saliva, the authors did not compare the individual results with matching serum specimens.

INDICATIONS FOR SALIVA TDM

There are numerous indications for saliva AED TDM, and these are similar to those for serum AED TDM[1]:

Dose Optimization on the Initially Prescribed AED

Measurement of saliva concentrations of the initially prescribed AED can be of particular value whenever the best therapeutic response has been achieved in an individual and maintained for a sufficient period of time to be confident that dosage has been optimized (ideally this would be seizure freedom but for many patients it would entail optimum seizure control with minimal and tolerated adverse effects). In this situation, measuring the saliva AED concentration at a standardized sampling time will allow identification of the "individual therapeutic concentration," which will be useful to interpret the clinical situation should a change in response occur during further follow-up.[1] The advantage of the "individual therapeutic concentration" approach is that it does not rely on fixed "reference ranges" and can be applied to any AED, including all the new AEDs for some of which "reference ranges" have yet to be clearly defined. Ideally, when establishing the individual therapeutic concentration, 2 separate determinations obtained at intervals of 2–4 months will be preferable to a single concentration measurement, because this allows an estimate of any variability.

Uncontrolled Seizures

Knowledge of the individual therapeutic concentration can greatly enhance management in patients who develop breakthrough seizures after a prolonged period of seizure control. For example, after a breakthrough seizure has occurred, if the salivary concentration is much lower than the previously determined individual therapeutic concentration it would suggest either suboptimal compliance or a clinically important change in the pharmacokinetics of the AED.[51,187] In the setting whereby seizures persist despite an apparently adequate dosage of an appropriate AED, saliva AED concentration monitoring would be useful to identify potential causes of therapeutic failure which may result from poor compliance (typically characterized by variable saliva concentrations, which increase after supervised drug intake) or from poor absorption, fast metabolism or drug interactions (typically characterized by low saliva AED concentrations).

In a series of 95 subjects with uncontrolled seizures that were admitted to an emergency department, saliva AED concentrations were able to reveal that noncompliance was responsible for the seizures in 31% of patients.[188] Zysset et al[189] in a series of 13 children with epilepsy observed that salivary phenytoin concentrations agreed well to that of clinical judgment about compliance and Herkes and Eadie[138] were able to identify noncompliance as a contributor to uncontrolled seizures in their patients by salivary TDM.

Suspected Toxicity

If toxic symptoms are suspected in a patient, saliva AED concentrations can aid in confirming a diagnosis of drug toxicity. For example, Cohen et al[120] showed a significant correlation between saliva concentrations and toxicity for phenytoin, whereas Hamilton et al[190] showed that carbamazepine saliva concentrations were significantly associated with adverse effects. A study of the incidence of gingival overgrowth in patients with epilepsy was undertaken to investigate whether there is a relationship between saliva (and serum) phenytoin concentrations and the extent, or prevalence of phenytoin-induced gingival overgrowth; there was none.[191] Fifty children receiving carbamazepine monotherapy were tested on a battery of cognitive and motor tests, and it was observed that peak and trough salivary carbamazepine

concentrations were related to several of the test variables.[192] Saliva AED monitoring is particularly valuable in patients whose clinical status is difficult to assess, for example, young children and subjects with mental and/or physical disability. In a series of 175 patients with epilepsy and mental disability, phenytoin and carbamazepine salivary concentrations were observed to exceed the therapeutic ranges of the 2 drugs on numerous occasions and the authors suggest that regular saliva TDM, along with neurological assessments should occur so as to avoid the possibility of toxic drug concentrations in this population.[193]

Recently, the pharmacokinetics of carbamazepine in saliva and serum were observed to be similar in a series of 20 patients experiencing acute poisoning with carbamazepine and who had been admitted to an Emergency Toxicology Unit.[194] The authors conclude that saliva carbamazepine monitoring can be usefully applied in managing patients experiencing acute carbamazepine-related toxicity.

Children

The serum clearance of AEDs in children is higher than adults and also age dependent. Children may require a weight for weight dose that is 2–3 times higher than that required to achieve the same drug concentration in an adult. Furthermore, clearance decreases gradually throughout childhood, but the precise time course of this process is not well established and is characterized by pronounced interindividual variability.[195] Thus, dosage requirements for children are constantly changing and are less predictable than in adults; therefore, AED concentrations are particularly helpful for optimal management.[196,197] Furthermore, most new AEDs are not initially approved for use in children, but many clinicians are prepared to prescribe them off label provided TDM is available. Because of the greater need to monitor AED concentrations in children, there have been many studies of saliva AED TDM in this patient group.[145,198,199] It is concluded that because saliva sampling is often preferred to blood sampling by children and for a child who is frightened by the sight of needles or by the prospect of having blood drawn, saliva is an acceptable alternative specimen. In addition, for preterm infants, who have very little blood volume and because venepuncture is an invasive procedure in these infants, the use of saliva for TDM would be advantageous; indeed such an approach has recently been described for caffeine monitoring.[200]

Pregnancy

During pregnancy, physiological changes occur that can alter drug absorption, distribution, metabolism, and elimination.[201,202] Therefore, careful adjustments to drug dosage may be required throughout the pregnancy to improve the efficacy and safety of prescribed medication. Gastrointestinal function can be prolonged (particularly during the third trimester) and as gestation advances the quantity of total body water and fat increases, which often increases the volume of distribution, causing drug concentrations in serum to decrease. Changes in cardiac output, ventilation, and renal/hepatic blood flow also occur while at the same time protein concentrations in serum decrease which results in the free (pharmacologically effective) concentration of drug increasing. Renal blood flow and glomerular filtration rate also change, which will affect serum concentrations of drugs predominantly eliminated unchanged by the kidneys. The increased secretion of estrogen and progesterone in pregnancy affects hepatic metabolism of drugs in different ways; such that pregnancy can alter the metabolizing capacity of hepatic enzymes. In addition the placental transport of drugs and their compartmentalization in the embryo/placenta along with metabolism by the placenta/fetus can play an important role in modifying the pharmacokinetics of a drug during gestation.

Thus, the pharmacokinetics of many AEDs are altered by pregnancy and most commonly serum concentrations decline (sometimes rapidly) throughout gestation; however, the extent of this effect varies with different AEDs and between patients.[203] The decline in serum AED concentration may be insignificant in some patients and pronounced in others, requiring dosage adjustments to maintain seizure control. Monitoring drug concentrations is therefore recommended during pregnancy and the usefulness of salivary monitoring has been well documented.[74,138,204] Salivary AED monitoring is particularly valuable for those drugs where the concentration in saliva reflects the non–protein bound quantity in serum and also when frequent TDM is recommended such as patients that are sensitive to small changes in their AED concentrations.

Elderly

Advancing age alters not only the way the body absorbs, binds, and eliminates drugs but also the way it responds to drugs.[195,205] As age increases, there is substantial interindividual and intraindividual variability in the pharmacokinetic changes resulting in large differences in the relationship between drug dose and serum concentration. The greater pharmacodynamic sensitivity observed in the elderly affects the response to any given serum concentration, which complicates interpretation of TDM results.[206]TDM of AEDs in the elderly is particular helpful for identifying noncompliance. Suboptimal compliance for example, underdosing, overdosing, missed doses, or make-up doses are common in older patients and affect serum drug concentrations and potentially the clinical response. Comorbidities and drug polytherapy are increased in the elderly compared with that in other age groups; therefore, pharmacokinetic interactions are more likely to occur and TDM helps safeguard against these situations.[205] Because albumin concentrations are decreased in the elderly, measurement of free non–protein bound drug concentrations is indicated for highly protein-bound AEDs,[207] and thus, salivary monitoring will be more helpful where the concentration in saliva reflects the free non–protein bound concentration in serum. However, for any AED where salivary concentration is correlated to those in serum, saliva can be used as an alternative sample and may be preferred when venous access is difficult as can occur in children and the elderly.[208] There have been 2 studies of the use of saliva AED measurements in the elderly whereby phenytoin was observed to correlate significantly with that of free non–protein bound phenytoin serum concentrations.[209,210]

Pathological States

Illnesses such as hepatic or renal failure, infections, burns, stroke, cardiac failure, human immunodeficiency virus

infection, and other conditions can significantly affect the absorption, distribution, elimination, and protein binding of AEDs.[142,211–215] In addition to the alterations caused by the pathological state per se, drugs used to treat these conditions can cause interactions that also affect AED concentrations; therefore, TDM should be employed in these situations.

Whenever a concurrent condition is known or suspected to alter protein binding, for example, renal or renal failure and after dialysis or surgery, when hypoalbuminemia occurs, or in patients receiving drugs that compete for protein binding sites, for example, aspirin, naproxen, tolbutamide and phenylbutazone[216] measurement of non–protein bound drug concentrations is essential, particularly for extensively bound AEDs. Salivary and serum phenytoin concentrations were measured in 7 patients with renal failure where salivary phenytoin concentrations closely correlated with saliva concentrations, and it was concluded that phenytoin therapy could be more appropriately monitored by measurement of salivary rather than serum drug concentrations.[142] Additionally, it was suggested that avoidance of blood sampling would be an advantage in uremic patients.

Hepatic disease can significantly alter the clearance of AEDs that are metabolized in the liver.[217] Furthermore, as the liver is the source of many proteins, serum protein binding may also be affected in hepatic disease and because it is impossible to predict the extent of change in AED clearance.[217] AED TDM (with unbound concentrations for highly bound drugs) is considered essential in patients with hepatic disease. Although there are no reports of salivary TDM of AEDs in hepatic disease this approach has been evaluated and proved useful for other drugs.[218]

Pharmacokinetic Interactions

In patients with refractory epilepsy, multiple drug therapy is common and monitoring serum AED concentrations is useful in identifying pharmacokinetic drug interactions and for making appropriate dosage adjustments. If patients treated with multiple drug therapy exhibit signs of toxicity or experience breakthrough seizures, AED TDM can help to ascertain which drug is more likely to be responsible for the patient's change in clinical status.[219,220] If an interaction is anticipated, it is good practice to measure a baseline drug concentration before adding the new drug.[221] Further TDM should be untaken at appropriate times after the potentially interacting agent has been added, and based on the result, the dose of AEDs can be adjusted accordingly. Salivary AED monitoring has been applied to quantify pharmacokinetic interactions in patients on polytherapy regimens. Interactions investigated include the serum protein binding displacement interaction between phenytoin and aspirin,[222] phenytoin and valproate,[76,223–226] and between phenytoin and azapropazone.[227] The metabolic Interaction between phenytoin and erythromycin has also been investigated.[228]

Changes in AED Formulation/ Generic Substitution

When the AED formulation prescribed for a patient is changed, for example, when switching to/from generic formulations or to/from an immediate-release to a sustained-release formulations, measuring an AED salivary concentration before and after the change is good practice because it identifies any differences in bioavailability, which would alter the steady-state concentrations and perhaps cause a change in the clinical status of the patient (eg, breakthrough seizers or exacerbation of adverse effects).[229–231] In some instances, collection of $\geq$2 saliva samples at different intervals after drug intake may be desirable to fully assess any change is absorption rate.

AED Pharmacokinetics

Because saliva can be collected repetitively and with ease over prolonged periods of time, it can provide a useful matrix to determine the pharmacokinetic characteristics of an AED and to compare the pharmacokinetics of different formulations. A number of such studies are reported, including that of lamotrigine in healthy volunteers[121] and patients with epilepsy[119] and carbamazepine and carbamazepine epoxide in children.[86,232] Additionally, the serum clearance of phenytoin and ethosuximide was reliably estimated based on a single measurement of phenytoin or ethosuximide in saliva.[233] More recently, the saliva pharmacokinetics of lacosamide were reported to be similar to that of serum and that this was the case when tablet and syrup formulations were compared.[234]

Eeg-Olofsson et al[52] in an open, controlled, within-patient study of 12 children were able to show that saliva and serum concentrations of both carbamazepine and carbamazepine–epoxide were related and used saliva to compare diurnal concentrations of both moieties after ingestion of 2 different carbamazepine formulations (Tegretol [Novartis, Philadelphia, PA] conventional carbamazepine versus Tegretol slow-release formulation). Hirji et al[235] similarly compared the saliva pharmacokinetics of phenytoin after ingestion of 5 different phenytoin formulations in 8 healthy volunteers and showed no significant difference in mean T_{max}, C_{max}, and area under the concentration versus time curve values. Finally, the relative bioavailability of carbamazepine of 4 different immediate-release carbamazepine formulations were ascertained in a series of 10 healthy volunteers by use of saliva carbamazepine measurements.[236] It was concluded that saliva is a suitable biological matrix in relative bioavailability studies.

SALIVA SAMPLING CONSIDERATIONS

Saliva Collection

Most people produce 1–1.5 L of saliva per day (although some produce larger quantities), and it can either be collected as it is produced naturally by expectoration into a suitable bottle or drawn from under the tongue with a needleless syringe and transferred into a tube. Alternatively, salivary flow can be stimulated by requiring the patient to chew an inert parafilm ball or by putting a drop of citric acid onto the tongue. Stimulated saliva is generally less viscous and so easier to analyze, whereas unstimulated saliva may require centrifugation because it often contains debris from the mouth.

In general, it is best to collect a volume of 2–3 mL, although most analytical methods require a lesser volume. As with blood samples, one should wait until steady state has been achieved before collection (except when acute or

transient toxicity is suspected). The use of stimulated saliva has several advantages over resting saliva: (1) a larger volume of the sample is obtained; (2) the pH gradient between serum and saliva is smaller and the variability in saliva/serum concentration ratios of some drugs is narrowed; and (3) it allows collection of specimens that are not viscous or discolored and therefore drug analysis can be undertaken more readily.[173]

Stimulating saliva changes the pH, and this can alter the distribution of the drug into saliva, particularly with basic drugs. It is necessary to validate the collection procedure and prove that the salivary excretion does not change as the sample is collected. This can be accomplished by undertaking 10 successive 1-mL saliva samples and testing the first, fifth, and tenth aliquots and if the drug concentration in each aliquot is similar, it can be concluded that the change in pH does not alter salivary excretion.

Saliva Collection Devises

There are a number of commercial saliva collection devices some of which contain a microfiber pad impregnated with salts (Cozart collector, Draeger drug test, Intercept, Oral Screen, OralLab, Quantisal, Omnisol, OraSure, SalivaScreen, Toxiquick, Salivette, Statsure, and Sorbette). With the exception of Omnisol and OraSure, these devices are not primarily aimed at the TDM market and they often use a proprietary diluent to mix with the collected saliva. Typically, the absorbent pad that is used to collect the saliva is added to the diluent, and thus, the precise volume of saliva collected is not known, which makes the devices unsuitable for quantitative analysis. With some devices, the saliva can be squeezed from the pad and sampled for quantitative analysis; however, most of the absorbing pads contain salts and salivary flow stimulants. These commercial devices need to be evaluated/validated for application to TDM of AEDs to demonstrate that the drugs are not irreversibly bound to the device.

One such study entailed the use of the OraSure and Omnisol devices whereby the feasibility of collecting saliva for TDM from children using these salivary collecting devices was evaluated.[237] There was no significant correlation between serum and saliva AED concentrations of phenobarbital, phenytoin, or carbamazepine. However, collection of naturally produced, liquid saliva in the same trial produced high correlation values for all 3 drugs (r^2 = 0.981, 0.976, and 0.888, respectively). In contrast, in a series 48 adult patients receiving phenytoin, a significant correlation (r^2 = 0.947) was observed between serum and saliva phenytoin concentrations when saliva was collected by the OraSure device.[238]

There are some commercial systems available, which collect a liquid saliva specimen:

1. *Greiner Bio-One*: This consists of 4 steps. First, the mouth is rinsed with a proprietary cleansing solution; second, the mouth is rinsed for 2 minutes to collect the saliva with a proprietary solution containing a dye; next, the mixed saliva/dye solution is dribbled into a collection beaker; and finally, the sample is aspirated into a vacuum collection tube. The dye allows a spectrophotometric evaluation to be undertaken to calculate the volume of saliva collected.
2. *Salicule Saliva collector*: This is a small, portable collector and dispenser that sucks an undiluted liquid saliva specimen from the oral cavity through a self-extendable tube for expectoration and into a graduated vial.

All commercial devices will require validation for each of the AEDs, which might be subject to salivary determination to demonstrate that the drugs are not irreversibly bound to any part of the device and also to ensure that nothing is introduced during sample collection, which interferes with the analytical procedure. Of course, there is a substantial cost associated with such collectors and various "home-made" products have been used and evaluated over the years including a spherical pouch device, which encloses sucrose so as to collect clean ultrafiltrate of saliva.[239,240] Another approach consists of a cotton wool ball wrapped in gauze, which is securely attached to a string. After absorption of saliva, the collector is placed into the barrel of a 20-mL syringe and squeezed out.[92] The former device was validated for carbamazepine and phenytoin, whereas the later device was validated for carbamazepine, carbamazepine epoxide, phenytoin, and phenobarbital.

Precautions for Saliva Sampling

During the collection of saliva, there is risk of spurious analytical results due to contamination from unhealthy gums and dental caries.[241,242] Ideally, a predose morning steady-state sample should be collected, which also gives the maximum time for saliva to flush the mouth. However, it has been demonstrated that saliva can be contaminated for at least 2 hours after holding a carbamazepine tablet in the mouth for just 5 seconds.[243] Similarly, phenytoin may persist for up to 3 hours after ingestion.[244] This clearly might impact on the TDM data; furthermore, if the patient is a tablet chewer or is prescribed a chewable or a liquid formulation and has dental caries, then pockets of drug can be deposited, which could contaminate the saliva sample and give a spuriously high result for a considerable period of time after drug ingestion. Also, if a liquid preparation is prescribed and saliva collected within a couple of hours of the dose, a false high concentration may result.[66] Finally, unhealthy, bleeding gums (gingivitis and gingival hyperplasia, which is common in patients prescribed phenytoin) would contaminate the saliva specimen with blood, and it may not be possible to obtain a satisfactory salivary measurement in such cases.

Saliva Sample Information

For salivary AED TDM to have maximum utility knowledge of sampling time and a meticulous dosage history is imperative. Sampling should occur at a steady state, which occurs at 5 half-lives (half-life values are shown in Table 4) after starting treatment or changing the dose. For AEDs with long half-lives (eg, ethosuximide, phenobarbital, phenytoin, zonisamide), the fluctuation in serum (and salivary) drug concentration during a dosing interval is negligible, and samples can be collected at any time. For the majority of AEDs that have shorter half-lives (eg, carbamazepine, eslicarbazepine acetate, gabapentin, lacosamide, levetiracetam, oxcarbazepine, pregabalin, retigabine, rufinamide, stiripentol, tiagabine, valproic acid, and vigabatrin), it is important to standardize sampling time in relation to dose. If saliva sampling is undertaken before reaching steady state so that the true steady-state

saliva concentration is underestimated, any dose increase that is implemented may result in toxicity for the patient. For carbamazepine, it is imperative that autoinduction is allowed to complete otherwise an overestimation of the steady-state concentration will occur, which may result in a dose that is subtherapeutic and patients may continue to have unnecessary seizures. The ideal saliva sampling time for all AEDs is in the morning immediately before the next oral dose (trough), but when this is not possible, for example, when attending an outpatient clinic, the sampling time and the time medication was last ingested must be recorded and the information sent to the laboratory with the specimen. If transient drug concentration–related toxicity is suspected, saliva should be sampled at the time the patient is experiencing adverse effects.

Saliva Sample Dispatch to the Laboratory

The collection of saliva is simple, noninvasive, and does not require a phlebotomist; therefore a saliva specimen can be collected by patients themselves or by their carers in the home environment and posted to the hospital laboratory. This approach has several advantages and provides opportunities for enhanced patient care as follows: (1) Samples can be collected at trough (ie, after an overnight fast and before the morning AED ingestion), which is the ideal time that relates to laboratory references values, (2) samples can be collected at a time when patients are experiencing adverse effects or breakthrough seizures which allows a drug-related effect to be established, (3) samples can be collected serially to evaluate the pharmacokinetics of a particular AED in a patient, (4) a saliva specimen can be posted to the hospital laboratory in advance of the patient's attendance at the clinic thus allowing the analytical report to be ready at the time of consultation which improves optimization of patient care.

For salivary drug tests that are to be posted to the laboratory, it is also necessary to establish stability of the sample under postal conditions, and this has been evaluated for some AEDs including carbamazepine, gabapentin, lamotrigine, levetiracetam, oxcarbazepine, phenytoin, phenobarbital, topiramate, and zonisamide.[93,114,186,245] The median time between saliva collection in the home and receipt in the laboratory was 4 days (range 1–160 days) in the study of gabapentin, lamotrigine, levetiracetam, oxcarbazepine, topiramate, and zonisamide, and all AEDs were observed to be stable.[186] Similar results were observed by Tennison et al[245] in their study of carbamazepine, phenytoin, and phenobarbital whereby the mean time between saliva collection in the home and receipt in the laboratory was 6.5 days (range 1–20 days). The stability of the other AEDs has yet to be investigated in this setting.

Financial Implications

Saliva sampling does not require a phlebotomist or a nurse to be available and requires less costly supplies (eg, needles, blood collection tubes, gauzes). The patients who benefit most are those that have a reduced cost of time from work, school, and travel, particularly those that must travel significant distances to the clinic or laboratory. One economic study compared the cost of saliva monitoring with that of blood monitoring in cooperative and uncooperative children and reported that for every 1000 samples in which saliva rather than blood was collected, there would be a cost saving of Canadian $1567 (55.4%) for cooperative children and Canadian $1822.25 (64.4%) for uncooperative children.[246]

Patient, Parent, and Physician Preferences

Chee et al[92] reported that in their series of 40 children aged 2–15 years, each child and parent were asked to complete a questionnaire indicating their preference for blood or saliva sampling without giving reasons for their preference. Of the 29 children and 30 parents who had completed the questionnaire, 66% of children and their parents preferred saliva sampling to blood sampling. The 24% who preferred venepuncture had been on chronic AED treatment for many years and were used to having blood taken by skilled staff. Gorodischer et al[246] investigated the preferences of 138 children and their parents with regards to saliva versus blood sampling. Although 117 of parents preferred saliva sampling (3 preferred blood sampling and 1 was indifferent), only 48 children preferred saliva sampling with 37 preferring blood sampling and 12 were indifferent. Interestingly, children who preferred saliva sampling were those who said they did not like blood tests, whereas most that preferred blood sampling were those who said that venepuncture did not hurt as they were already used to it. In the study conducted by Tennison et al[245] comprising 102 patients of mean age of 27.5 years (range 1.6–89.9 years), with 42 being children less than 18 years old, all parents and most children preferred saliva collection, probably because of the painless nature of the process. Of the patients studied, 32 preferred home saliva sampling, 5 preferred blood, and 24 had no preference. Furthermore, the majority of collectors rated saliva no more difficult to collect than blood.[245] Knott and Reynolds[50] also highlighted that in their clinical experience most of their patients, particularly the children, preferred saliva to blood sampling.

A survey of 945 child neurologists of whom 58% (544) returned the questionnaire indicated that only 0.4% had requested a saliva AED concentration in the last year, and only 6% (33/544) indicated that saliva concentrations were available to them.[247] What was particularly informative was that 286/522 (55%) of responders thought that AED concentrations in saliva achieved by a painless method as opposed to serum, which require venepuncture, would be valuable to the care of their pediatric practice. Indeed the potential to obtain an immediate sample at home for AED concentration determination at the time of a seizure or adverse effect was considered to be of value by 70% of physicians (370/526). Finally, it has been estimated that 3.5% of patients have an injury-injection phobia and salivary monitoring would also eliminate any risk of infection related to venepuncture.[248]

ANALYSIS OF AEDS IN SALIVA

The analysis of AEDs in saliva can be undertaken using the same basic analytical methods that are applied to plasma/serum. These include immunoassays and a range of chromatographic procedures.[53,122,191,249–252] However, it is vital that each and every analytical method is fully validated for use with saliva

before application with this biological matrix. The validation should be rigorous and similar to that undertaken for serum.

Compared with serum measurement, some important issues with respect to salivary AED determination will be the range of calibration, sensitivity requirements, and quality control. Because the salivary concentration is similar to the non–protein bound concentration for most AEDs, the range of calibration will need to be considerably lower for the extensively bound drugs, for example, phenytoin. Furthermore, with chromatographic methods, it may be necessary to sample a larger aliquot for saliva compared with serum to achieve adequate sensitivity. For immunoassays, it would be necessary to adapt the operating conditions and extend the incubation times to move into a lower calibration range.

Because some saliva can be extremely viscous, it is very important that procedures are in place to ensure it is sampled accurately; also, additional steps may be required to ensure that residual food, etc, does not interfere with the sampling process.

Each analytical run should contain internal quality control specimens at 3 concentrations, which span the range of calibration. These QC specimens should be prepared in saliva (as should the calibrators unless an alternative matrix has been tested and shown to be equivalent). At the present time, the External Quality Assurance schemes for AEDs do not circulate salivary specimens but hopefully will begin to do so once the TDM of AEDs in saliva becomes more widely used as an alternative to serum.

PRACTICAL PROCEDURE FOR SALIVA AED TDM

For saliva to be useful for AED TDM, there must be a consistent relationship between serum and saliva drug concentrations; this could be that saliva reflects total serum concentrations or ideally is equal to the free, pharmacologically active, concentration in serum.

Ten minutes before collecting saliva, patients should rinse their mouths with plain water to reduce potential contamination with drug or food particles. With infants, uncooperative children and patients with learning disability the oral cavity can be rinsed by spraying with water from a syringe. If there is evidence of particulate matter, abnormal discoloration (eg, blood contamination) or the saliva sample is abnormally viscous, it should be discarded and a fresh specimen collected. Recollection should occur after rinsing the patients' mouth with water and waiting 15 minutes.

Collection of Saliva Samples

1. The ideal sampling time is after an overnight fast and just before drug ingestion or just before the ingestion of the next scheduled drug dose. If this is not possible, the specimen should be collected at least 2–3 hours after drug ingestion.
2. In the case of transient concentration-related toxicity, saliva should be collected at the time the patient is experiencing adverse effects.
3. If possible, stimulate salivary flow by chewing of an inert material such as paraffin wax (Parafilm). Citric acid stimulation can be achieved by placing approximately 10 mg of citric acid crystals or a drop of lemon juice on the tongue.
4. Saliva can be collected either by expectoration or aspirated with a syringe from the buccal cavity and deposited into a suitable container or tube.
5. The saliva collected during the first 2 minutes should be discarded.
6. The collected specimen (1–2 mL) should either be stored frozen until dispatch to the laboratory for analysis or dispatched on the same day. The specimen will remain stable for several days at ambient temperatures.

Sample Information

For the sample to be processed efficiently and for the results to be interpretable, it is important that the following information is provided on an appropriate AED TDM Request Form: Patient name, sex, and date of birth; AEDs for which analysis is requested, including prescribed dose(s), information regarding all other drugs prescribed; time of saliva sampling; time of drug ingestion; indication/reason for TDM.

Dispatch of Saliva Samples

Samples (unfrozen) should be dispatched by first class post, using packaging currently recommended for transporting pathological samples by post. Alternatively, they can be sent to the laboratory by courier at ambient temperature.

Receipt of Samples in the Laboratory

Upon receipt of samples in the laboratory, samples should be transferred to a centrifugation tube and the specimen centrifuged to obtain a clear supernatant, which can subsequently be analyzed for AED content. Thick and grossly clouded or discolored supernatants should be rejected.

Patient Report

For clinicians, salivary concentrations may be somewhat confusing because they are trained to understand and interpret serum concentrations. Although reference ranges have been suggested for a limited number of AEDs (eg, carbamazepine, phenytoin, and phenobarbital), they have not been fully validated.[91] Therefore, to aid interpretation, the following procedure is followed in the authors' laboratory: The laboratory-generated report should contain all the sample information highlighted above along with the saliva concentration measurement of the requested AEDs. For AEDs whose salivary concentrations are equal to that of the free non–protein bound concentrations in serum (eg, carbamazepine, phenytoin), an additional comment is included, which indicates the calculated serum concentration equivalent. For example, "this saliva concentration is equivalent to a total serum concentration of ???".

SUMMARY AND CONCLUSIONS

TDM using serum/plasma concentration of AEDs is a well-established means of optimizing epilepsy treatment, and guidelines are published, which are aimed to ensure proper use of TDM.[1] Although TDM is mostly undertaken in serum, many AEDs can be readily monitored using saliva. For highly protein-bound AEDs, saliva has the advantage of

reflecting the free non–protein bound pharmacologically active concentration of drug in serum. The clinical value of monitoring free non–protein bound drug concentrations is particularly applicable to AEDs such as phenytoin and valproic acid, which are >90% protein bound.[253] However, because all AEDs are either hepatically or renally excreted hepatic and/or renal disease will impact on their protein binding and thus make salivary TDM particularly useful.

The primary requisite for salivary monitoring to be of value is a constant or predictable relationship between the drug concentration in saliva and the drug concentration in serum. For many AEDs (carbamazepine, clobazam, ethosuximide, gabapentin, lacosamide, lamotrigine, levetiracetam, oxcarbazepine, phenobarbital, phenytoin, primidone, topiramate, and zonisamide), there is compelling evidence that saliva can be a useful alternative to serum for individualizing the drug treatment of patients with epilepsy. However, salivary TDM of valproic acid is probably not helpful, whereas for clonazepam, eslicarbazepine acetate, felbamate, pregabalin, retigabine, rufinamide, stiripentol, tiagabine, and vigabatrin the data are sparse or nonexistent.

Salivary sampling is convenient, painless, and a noninvasive alternative to serum monitoring of AEDs. It is especially suitable for TDM in both children and the elderly, also in those with needle phobia. The collection of saliva can be performed with a minimum of instruction, does not require a professional phlebotomist, is painless, incurs minimal cost, and is widely accepted by patients and physicians. Salivary TDM will enable new approaches to treatment with strategic at-home monitoring either at the time a seizure/adverse event occurs or allowing more routine samples to be collected by a care giver and mailed to the laboratory so that the result is available in advance of a clinic visit. Furthermore, salivary collection can be easily repeated on a daily basis if necessary to investigate a specific clinical question or manage an intractable situation, for example, to undertake dosage adjustments for optimizing therapy in patients with poor seizure control; in pregnant women when the time course of the change in drug concentrations relative to dose can be followed more closely throughout pregnancy and the postpartum period than is practical when using serum concentration measurements.[138]

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