Chapter 12

Therapeutic Monitoring of Anticonvulsants: Use of Saliva as Biological Fluid

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Abstract

Plasma drug concentration is not homogeneous within the intravascular space, being the arterial (P_A) concentrations higher or lower than that in veins (P_V) depending on whether the samples are taken during the drug absorption or the elimination phases, respectively. However, blood samples are currently withdrawn from peripheral veins and total (bound plus unbound) plasma drug concentration is assayed. Despite the fact that free plasma drug levels (P_{fV}) are not determined in routine therapeutic drug monitoring (TDM), they could be assayed for research purposes. Salivary drug concentrations (S) approximate to their free plasma levels in the arteries of the great circulation. Saliva is recommended to be collected with stimulation to minimize the difference between the pHs of both fluids (saliva and blood), and thus, artery/vein-free drug concentration ratios (P_{fA}/P_{fV}) could be surrogated by the stimulated saliva/free-plasma in vein drug concentration ratios (S_S/P_{fV}). It is possible in this way not only to assess this S/P ratio but also to infer the brain (B)/P_{fV} ratio, which is actually the most relevant for antiepileptic drugs (AEDs). Different cases of AEDs are considered in this review, taking into account their physiochemical properties and their ability to be transported by membrane carriers.

Keywords: drug concentration in saliva, saliva-to-plasma concentration ratio, brain-to-saliva concentration ratio

1. Introduction

The concept of therapeutic drug monitoring (TDM) in plasma or serum of antiepileptic drugs (AEDs) is led by the assumption that the pharmacodynamic effects of drugs correlate better with circulating concentrations than with administered doses. TDM encompasses both drug

quantification in a sample and pharmacological interpretation for dosage adjustment. Although TDM has been used as a tool to optimize treatment of epilepsy for almost 50 years, evidence for its usefulness in improving clinical outcome is scarce and controversial. The main potential pitfall regarding TDM interpretation is that we are still measuring drug levels in a fluid far away from the site of action. Plasma drug concentration is not homogeneous within the intravascular space, while all arteries have the same drug level value, each vein coming from different organs may have different drug concentrations, among them and in relation with their respective arteries. Arterial drug concentration is higher than the respective venous concentration during input of the active substance either after intravenous or oral administration. The opposite is observed when drug elimination predominates. This circulatory issue has been well referenced in the literature both in animals [1] and in humans [2], and gives evidence to understand the discrepancy between plasma venous drug concentrations, which are commonly measured, and drug effects [2, 3]. Blood samples are currently withdrawn from peripheral veins and total (bound plus unbound) plasma drug concentration is assayed although only the free drug is responsible for the pharmacological effect.

Measurement of AED concentrations in brain interstitial fluid (BIF) could be the solution as such concentrations are considered to reflect those occurring in the brain, which result in the pharmacological effect of the drug. Furthermore, BIF concentrations would reflect the free serum concentration. However, the impossibility of obtaining this fluid turns it in an inappropriate biological matrix for AEDs TDM purpose.

Saliva has been investigated by our group as an alternative biological fluid for TDM of AEDs. Saliva is produced in the salivary glands by ultrafiltration of arterial plasma. For this reason, of particular advantage, apart from the easiness to collect and the fact that saliva can be sampled repetitively, is that the concentration in saliva approximates to its free plasma levels in the arteries of the great circulation.

Saliva is recommended to be collected with stimulation to minimize the difference between the pHs of both fluids, and thus, artery/vein free drug concentration ratios could be surrogated by the stimulated-saliva/free-plasma-in-vein drug concentration ratios. It is possible in this way not only to assess this saliva/plasma (S/P) ratio but also to infer the brain/free plasma drug ratio, which is actually the most relevant for AEDs.

Different chemical structures and mechanism actions identify AEDs. Acting on ion movements (voltage-gated sodium and calcium channels) or postsynaptic receptors (gamma-aminobutyric acid and glutamate receptors) characterized first-generation AEDs. Second- and third-generation AEDS focused on ion movements but through different channels such as the neuronal KCNQ potassium channels [ritagabine (RTG)], or targeting the voltage-gated sodium channels but enhancing the slow inactivation of the channel [lacosamide (LCM)]. Some drugs were synthesized as GABA analogs [(gabapentin (GBP) and pregabalin (PGB)] but they do not act directly on GABA receptors. They bind to a subunit of presynaptic voltage-gated N-type Ca^{2+} channels, decreasing calcium entry and avoiding therefore glutamate release. Regarding their chemical structure, AEDs are carboxamide derivatives [phenobarbital (PHB), phenytoin (PHT), ethosuximide (ESM), carbamazepine (CBZ), oxcarbamazepine (OXC), levetiracetam (LEV), RTG, LCM], sulfonamides and sulfamates [felbamate (FBM), zonisamide (ZNS),

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topiramate (TPM)], amino acid compounds [vigabatrin (VGT), GBP, PGB], carboxylic acids [valproic acid (VPA), tiagabine (TGB)], and heterocycle amines [lamotrigine (LTG)].

In accordance with their chemical structures most of them are not ionized in body fluids, except for PHB, amino acid and carboxylic acid drugs, and LTG. Despite some extent of ionization, non-ionized moieties of AEDs have enough lipophilicity to cross the blood-brain barrier (BBB) rapidly. Plasma (P) and saliva (S) are the main biological fluids used for drug monitoring. Because of the lower pH found in saliva (6.4) than in plasma (7.4) [4] and the acidic characteristic of PHB, VGT, GBP, PGB, VPA, and TGB, a lower S/P concentration ratio than their respective free/total plasma concentration ratio is obtained [5]. Conversely, LTG has a preference for saliva due to its basic properties, and then a higher S/P than free/total plasma ratio can be foreseen. All the other antiepileptic compounds are expected to have similar S/P and free/total concentration ratios. However, not only pH-partition considerations have to be taken into account to forecast S/P concentration ratios.

The aim of this review is to discuss the potential use of saliva as a biological matrix to perform AED TDM.

2. Arteriovenous (A-V) difference in plasma drug concentration

During drug input, arteries have higher drug concentrations than all the veins of the large circulation, except for the vein through which the substance enters the body. So, while the drug is entering the body arteries are transporting an amount of substance that exceeds the one previously eliminated. This is repeated after each circulatory cycle until the steady state is reached. At this point, the amount of drug entering the body is the same as the one that is being eliminated, and the concentrations in veins and arteries become equal.

Once the administration is interrupted, drug decay proceeds from all branches of the circulatory apparatus. After drug input ceases, the veins exhibit higher concentrations than the arteries, except for those veins coming from eliminating organs. This inversion during the elimination phase is because the blood entering the arteries of the large circulation suffered a dilution caused by the lesser content of solute that veins coming from the eliminatory organs had.

It is important to bear in mind that not only the absorption or elimination of a drug rules the A-V difference in drug concentration but also changes in the migration of substances outside the vessels. These changes take place during physical activity of individuals and they could modify the A/V drug concentration ratio. A sudden increase in the distribution of cardiac output [6] to the muscles might force the drug to disappear from the intravascular space, rendering a decrease in the A/V drug concentration ratio at those organs not involved in the migration of solute. This situation reverts once subjects stop doing muscular activity, and the A/V ratio increases up to the previous value as if a process of drug absorption is operating from the muscles.

3. Saliva production

Saliva is the fluid produced by the salivary glands, and is made up mainly of water, electrolytes, mucus and enzymes. Humans have three major pairs of salivary glands that differ in the type of secretion they produce: 1—parotid glands, which produce a serous watery secretion; 2—submaxillary (mandibular) glands, which produce a mixed serous and mucous secretion; and 3—sublingual glands, which secrete saliva that is predominantly mucous in character.

The basic secretory units of salivary glands are clusters of cells called acini. These cells secrete a fluid that contains water, electrolytes, mucus and enzymes, all of which flow out of the acinus into collecting ducts. Within the ducts, the composition of the secretion is altered. Much of the sodium is actively reabsorbed, potassium and protons secreted, and large quantities of bicarbonate ion reabsorbed [7]. Small collecting ducts within salivary glands lead into larger ducts, eventually forming a single large duct that empties into the oral cavity.

Secretion of saliva is under control of the autonomic nervous system. Traditionally, acetylcholine is the parasympathetic postganglionic transmitter and noradrenaline the sympathetic postganglionic transmitter that act on the secretory elements of the glands. Noradrenaline acts on α1-adrenoceptors and β1-adrenoceptors, whereas acetylcholine acts on muscarinic M1 and M3 receptors. Parasympathetically induced vasodilatation may generate a 20-fold increase in gland blood flow, which ensures the secretory cells produce large volumes of saliva over a long period of time. The parasympathetic transmitter vasoactive intestinal peptide, besides acetylcholine, plays a major role in the vasodilator response, which also involves the action of NO. Stimulation of the sympathetic innervation initially causes vasoconstriction by α1-adrenergic receptors and then a vasodilatation mediated by β1-adrenoceptors increasing the gland blood flow.

4. Saliva drug concentration and saliva-to-plasma level ratio

Saliva has been used as an alternative biological fluid for TDM for more than three decades. Nowadays it is emerging again as a valuable matrix for AED TDM because it is associated with several advantages over the conventional sampling fluids: plasma, serum, or blood [5]. Nevertheless, few articles deal with the most relevant advantage that drug monitoring in saliva has [8, 9], that is, a measure of the drug directly available (free in the arterial plasma) for all body tissues, including the brain.

As other organs, salivary glands receive substances from the arterial part of capillaries and thereafter solutes are transferred through the basal and apical membranes of acinar cells into the upper zone of salivary ducts. The fluid recently formed in acini has a similar pH and a similar composition in free substances to the plasma in the artery [8]. This is true for substances which have no restriction in their passage through lipophilic membranes, such as AEDs. During its transit through the luminal space of ducts the fluid interchanges protons, other ions and solutes with the interior of ductal cells through the apical membrane, and thereafter with

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the interstitial space through the basal membrane [7]. So, drug molecules located in the arterial space of the circulatory system pass through the acini into the salivary conducts, returning back to the circulatory system through the veins from ductal cells.

When saliva is secreted into the oral cavity, drug concentration and pH differ sensitively from the value they have at the upper zone of ducts. Saliva becomes more acidic and more or less concentrated in AEDs depending on their physicochemical characteristics. The volume withdrawn determines whether the drug concentration in this fluid would be closer to the free plasma venous value or to the arterial one. For non-ionized AEDs, the smaller the volume of saliva is (usually obtained without stimulation, or the first fraction obtained after stimulation), the closer to free plasma venous concentration becomes. In the case of weak acid molecules, lower values than the corresponding free ones in venous plasma are obtained since the pH at the lower part of the ducts is more acidic than in blood (and in the acinus). Conversely, in the case of basic AEDs, a higher value than the free plasma venous concentration should be expected.

When saliva volumes are large, or when saliva is obtained with stimulation (chewing parafilm®, or putting small amounts of citric acid crystals on the tongue), saliva AED concentrations become closer to the upper part of the ducts (acini). As it was reported in the literature [10, 11], the variability in saliva drug concentration could be diminished by using stimulated saliva sampling.

Figures 1 and **2** show saliva collection without or with stimulation, respectively.

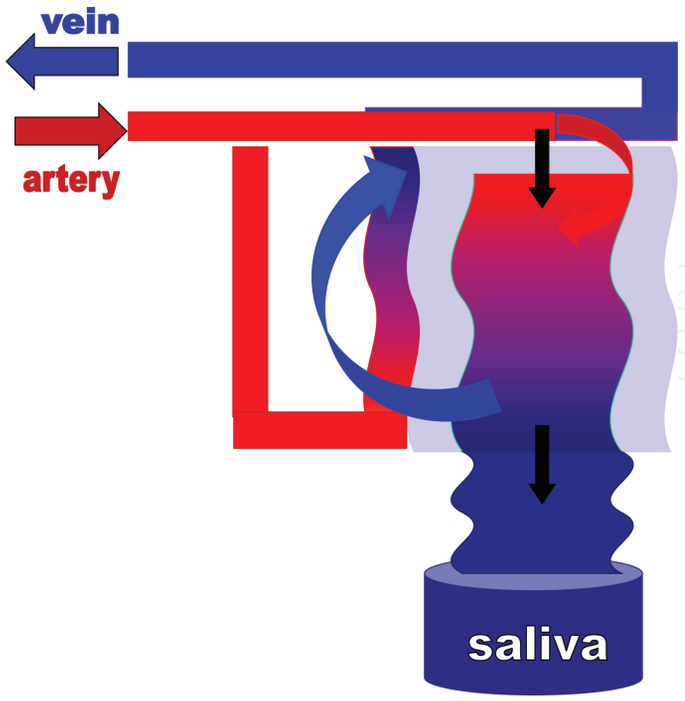

Figure 1. Schematic representation of saliva collection without stimulation.

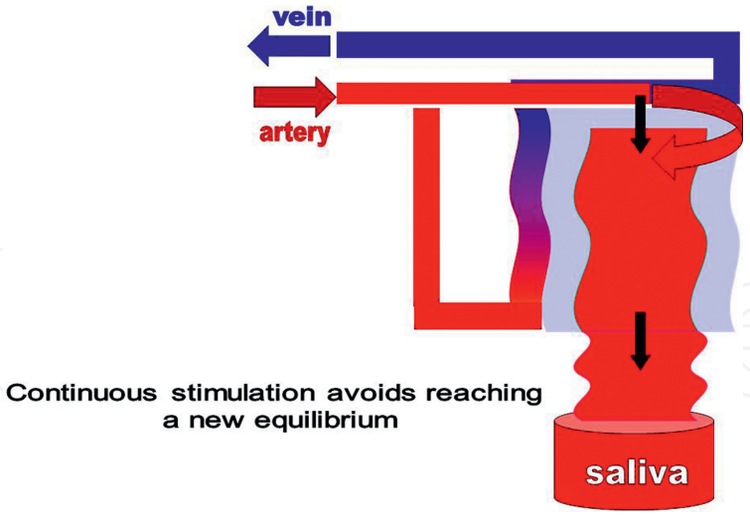

Figure 2. Schematic representation of saliva collection with stimulation.

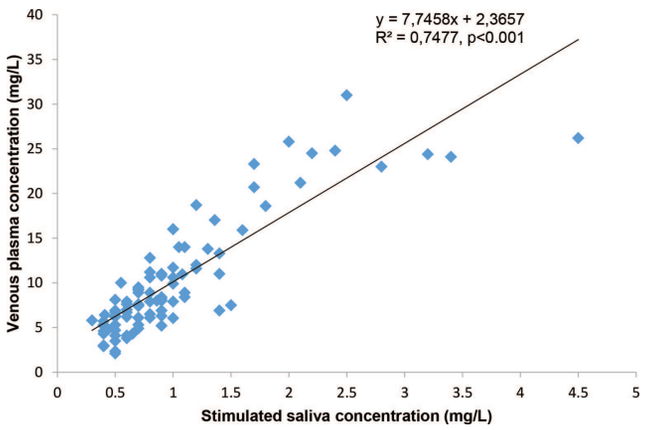

Figure 3. Predose venous plasma concentration versus predose stimulated saliva concentration in patients receiving PHT.

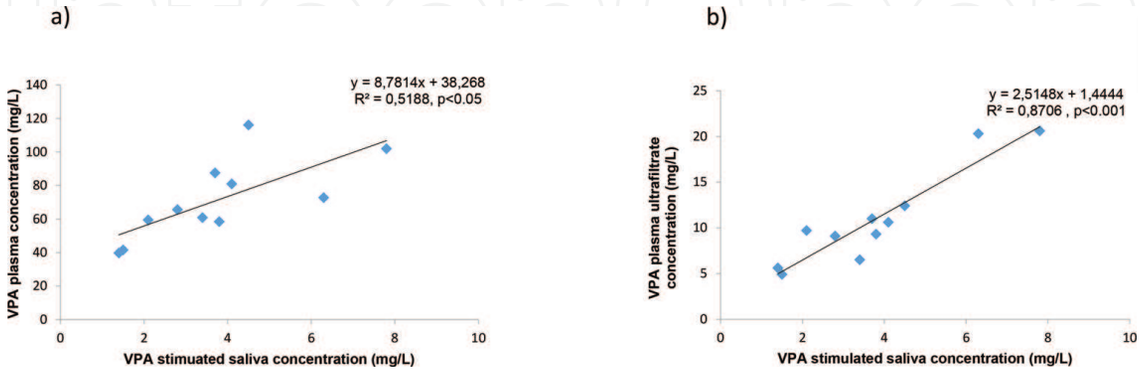

Figure 4. VPA plasma concentration versus VPA stimulated saliva concentration (a) and VPA plasma ultrafiltrate concentration versus VPA stimulated saliva concentration (b) in 11 pediatric patients.

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For a number of AEDS drugs, mainly those which are lipophilic and non-ionized at salivary pH range (i.e. PHT), stimulated or non-stimulated saliva concentrations highly correlated with plasma concentrations. **Figure 3** shows pre-dose venous plasma and citric acid-stimulated saliva samples obtained in 94 patients taking PHT for seizure control. For VPA, which is more ionized in plasma than in saliva, citric acid stimulation seemed to be adequate to diminish pH variability. A great volume of saliva was drained from salivary ducts and thereafter its pH became closer to blood pH. Stimulated saliva and blood samples (prior to the morning dose) were withdrawn from eleven children diagnosed with epilepsy receiving VPA as monotherapy. Interestingly, as it is shown in **Figure 4**, saliva concentrations correlated with plasma concentrations (**Figure 4a**, $p < 0.05$) but a higher correlation was found between saliva levels and VPA ultrafiltrate plasma concentrations (**Figure 4b**, $p < 0.001$).

It is also noteworthy that no matter the time after dose the samples were taken, during the absorption or the elimination phase, the stimulated saliva (S_S) drug level would always be linked with the free serum level at the arterial plasma.

Under this mode of sampling, the S_S/P concentration ratio should be understood as an approach for measuring the A/V drug concentration ratio since P levels usually come from venous blood specimens. Performed as such, saliva drug concentration would be closer to the free plasma drug concentration in all arteries (P_{fA}) of the great circulation and above (during the absorption), or below (during the elimination), free plasma level in the vein (P_{fV}) of almost all the organs that do not participate either in drug entry or exit from the body.

It is currently known that efflux pumps belonging to the ABC (ATP binding cassette) family of transporters, such as P-glycoprotein (Pgp) and multidrug resistance protein 2 (MRP2), are located at the apical membrane of both acinar and ductal cells [12]. Some AEDs are substrate of these transporters, and then, the S/P ratio could be effectively affected by changes in the activity or the expression of efflux carriers. Because of this, salivary levels were used for assessing the systemic modification in efflux transporters [13]. Pre-dose sampling is preferred to minimize the contribution of drug absorption in the S/P ratio. Significant increases in the pre-dose S/P concentration ratio for CBZ and PHT, throughout the time-course of their chronic administrations [14, 15] revealed their inductive effect over their own membrane transporters [16, 17].

On the basis of current literature data [2, 18] it is possible to retrieve the same conclusion issued by our group, since the S_S/P_{fV} ratios assessed for PHT and PHB, two recognized efflux transporter inducers, were above 1 (average value: 1.10 and 1.06, respectively). This ratio should be approximately 1 or below 1 if there is no drug entrance. Due to the higher clearance that CBZ has after chronic administration, pre-dose S_S/P_{fV} should be theoretically much lower than 1 [19], but because of its inductive effect [14] the obtained value resulted practically 1 [18]. It is important to remark that to reach a reliable conclusion about overexpression of efflux carriers, S_S/P_{fV} must be determined when drug absorption is not operating. If this is not considered, misleading results could be obtained. For instance, CBZ yielded higher S_S/P_{fV} ratios (1.39–1.44) when samples were taken from 1 to 5 h after dose intake [20]. These higher ratios are related more to the effect of drug absorption than to its inductive effect on efflux transporters.

On the other hand, drugs that are not recognized as inducers of membrane carriers, such as LTG or LEV, rendered S_S/P_{fV} values of 0.82 [21] and 0.36–0.41 [22] respectively.

Interestingly, LCM given to healthy subjects [23] or to controlled epileptic patients after a single dose [24] rendered S_S/P_{fV} lower than 1 during the elimination phase of the drug, with a consistent plasma protein binding of 15%. However, when LCM was given as adjunctive therapy to patients with intractable epilepsy [25], not only its protein binding increased to around 90% but also the S_S/P_{fV} rose to 1.44. In most of the patients, co-medications were efflux transporter inducers. This last fact could have resulted in a preferential transfer of LCM to saliva due to its affinity for Pgp [26]. It is known that seizures are associated with an increased inflammatory response [27], which in turn enhances alpha-1-acid glycoprotein (AAG) plasma levels [28] and overexpresses efflux transporters at the BBB and tissues far away from the central nervous system (CNS) [29]. This overexpression could reinforce the transport of LCM from blood to saliva. On the other hand, the increased protein binding rate of LCM in refractory epilepsy patients could be related to its eventual binding to AAG, but up to date this issue has not been studied.

5. Saliva-to-brain drug concentration ratio

The main objective in TDM is to follow-up patient´s pharmacotherapy by means of drug levels to predict the evolution of the treatment, or to interpret both the occurrence of adverse reactions and therapeutic failures; in other words, to infer drug concentration at its action site (in the case of AEDs, for effectiveness, at the brain). Which drug concentration, plasma or saliva, can be the appropriate as a surrogate for assessing CNS exposure? The answer to this question is not an easy issue, since no decision has been made for the best matrix to pursuit AED TDM so far. Past and current approaches were carried out just to correlate saliva with plasma AED concentrations. Maybe, this objective is substantially more affordable and suitable than the main issue, or perhaps because either with plasma or with saliva the main problem of refractoriness to AEDs still remains as a great challenge.

Strong evidence [29–33] supports the hypothesis of an overexpression of efflux transporter, not only in the brain but also in the rest of the body, caused by uncontrolled seizures.

As previously discussed, two scenarios can be possible: (1) the AED used in the treatment is not a substrate of efflux transporter or (2) it is a substrate. In the first case, pre-dose stimulated saliva is a valuable tool to follow-up the treatment of non-ionized AEDs that are not so highly bound to plasma protein. Advantages of saliva TDM of AEDs as an alternative to plasma TDM include: (a) sample collection is painless and non-invasive, (b) it is more economical and with reasonable sensitivity, specificity, accuracy, precision of the analytical methods. In the case of acidic ionized compounds the use of stimulated saliva increases its concentration up to the corresponding free serum value since saliva pH value rises and becomes comparable to that of plasma. A decreased salivary level for basic drugs would be attained. To sum up, AEDs that are not transported by efflux carriers would have in the brain the same levels as their stimulated salivary concentrations.

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Some considerations should be taken into account for AEDs substrate of efflux transporters to infer their concentrations in the brain. If the AED is an efflux transporter inducer, if the clinical response of the patient is worsening throughout the time course of the treatment, and if the response is not appropriately related with plasma or saliva drug concentration, a decreased brain concentration should be suspected. To corroborate this fact, pre-dose stimulated saliva and venous plasma samples should be taken and the S_S/P_{fV} evaluated. If this ratio is above 1, there is a great chance of developing refractoriness to the treatment. Discontinuation of the drug, or a change in its dosage regime [34] could be assayed.

When the AED is a substrate of efflux transporters but it does not induce them, such as LCM [26] and LTG [35], apart from following-up the anticonvulsant treatment through measurements of AED in saliva, the evolution of silent antiepileptic responses could be monitored by assessing the S_S/P_{fV} throughout the time course of the treatment. If the ratio lowers, the observed control of seizures might be additionally supported with a favorable prognostic. If the ratio tends to increase, dose reinforcement of the AED or some other therapeutic alternative should be considered.

In conclusion, in all the cases dealing with an efflux transporter substrate, an inverse relationship between brain-to-plasma and saliva-to-plasma would be inferred.

6. Conclusions

Salivary drug concentration measurement is an efficient clinical practice for monitoring epileptic patients. This cost-saving and easy-to-obtain fluid gives valuable information not only of the AED treatment but also of the clinical evolution of the epilepsy.

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