Introduction

Pregabalin is the structural analogue of, but functionally unrelated to, the naturally occurring transmitter GABA (gamma-aminobutyric acid). It is used as an adjunct treatment for focal epilepsies, neuropathic pain, fibromyalgia, and anxiety disorder in adult patients [1, 2]. Pregabalin binds to the α-2-δ subunit of voltage-gated calcium channels, decreasing the release of several excitatory neurotransmitters and blocking the development of hyperalgesia and central sensitization [3]. No clear reference range has been established [4].

Pregabalin is rapidly and extensively absorbed after oral dosing in the fasted state, with maximal plasma concentration occurring within 1 h after single or multiple doses, and steady state being achieved within 24–48 h after repeated administration. The oral bioavailability of pregabalin is high at ≥ 90 % and is independent of the dose. The mean elimination is mainly renal that depends on creatinine clearance and does not bind to plasma proteins [5–7]. In addition, the concentration–time profiles of pregabalin are similar after two or three times daily administration, which reflects the clinical findings that pregabalin administered via either dosing regimen resulted in similar efficacy [8].

Pregabalin also is not subject to hepatic metabolism and does not induce or inhibit liver enzymes such as the cytochrome P450 system. Therefore, pregabalin is unlikely to cause, or be subject to, pharmacokinetic drug–drug interactions, an expectation that has been confirmed in clinical pharmacokinetic studies. Features of minimal drug interaction potential are important for clinicians treating patients who are frequently on a number of different therapies, such as the elderly or those with refractory epilepsy. However, as expected from the importance of the renal system in the
elimination of pregabalin, both AUC and the elimination t1/2 increase with decreasing renal function so, dose reduction in patients with compromised renal function (i.e., patients with a creatinine clearance of < 60 ml/min) is needed [9].

For drugs that are highly bound to blood proteins, only the free fraction is pharmacologically active [10]. Saliva contains only the free fraction of drugs that could penetrate through the salivary tissues including the capillary wall, the basement membrane, and the membrane of the salivary gland epithelial cells [11]. In clinical conditions in which protein binding varies, drug concentration in oral fluid is more closely related to the therapeutically active fraction of drug than in plasma [11, 12]. Also, in cases where the concurrent use of two or more drugs may alter drug binding to plasma protein, the oral fluid concentration reflect the plasma free drug concentration. Therefore, saliva has been increasingly used for therapeutic monitoring of drugs as well as a diagnostic medium for the measurement endogenous markers [13–20].

Saliva can act as a diagnostic medium which provides many advantages over plasma. Saliva is a non-invasive specimen that obviously advantageous for obtaining samples from those whom, for cultural reasons or age or because of physical or mental handicaps, would be unethical to collect blood samples. The free, rather than the protein-bound drug molecules are considered to be the active component in blood. Thus, the drug levels in saliva are thought to reflect the free drug concentration. Hence, saliva efficiently reflects the drug activity. The rules of drug protein binding and membrane permeability on salivary excretion were previously investigated for the drug activity. The criteria that should be available in the selected patients include: current therapy with pregabalin and patients should be at steady state concentration for pregabalin (steady state is achieved within 1 to 2 days of repeated administration), consent form is available.

Four samples were taken from each patient, two samples of saliva and another two of blood. First sample of both saliva and blood is the trough sample and was taken just before the first dose of the day, second sample is the peak sample and was taken 1 h after taking the first dose of the day. The pregabalin products that the patient had taken (Lyrical, Zega, Neo Gaba, Nervax, Regab and Epigab). In addition serum creatinine was also measured.

**Assay methodology**

Blank human plasma and saliva samples were derived from healthy volunteers at Triumpharma LLC to establish and validate the method of analysis using LC-MS/MS (API 4000). Theophylline internal standard 30 μl (3.00 μg/ml) was added to 200 μl of subject plasma, vortex the samples for about 10 s, 2.0 ml of methanol was added, vortex the samples for about 10 s, 1.0 ml of mobile phase was added, vortex the samples for about 30 s, the samples were centrifuged at 10000 rpm for 5.0 min then 200 μl of the supernatant was transferred to labeled clean HPLC vials and the samples were placed in the auto sampler and 10 μl was injected. Calibration curves were constructed by plotting the analytical response of the instrument (Analyte area/IS area) versus pregabalin concentration. A linear relationship (R = 0.9985) between the analytical response and concentrations of pregabalin was obtained over concentration range (0.02–10) μg/ml with average recovery of 100%. The limit of detection and quantification is 0.02 ng/ml. method was linear in range of 20–4000 ng/ml, with inter and intra day variability of < 10%.

**Data analysis**

Calculated pharmacokinetic parameters

The following pharmacokinetic parameters of pregabalin were calculated:

- Creatinine clearance calculation: Cockcroft & Gault equation used to calculate creatinine clearance (7).

\[ Cl_{cr} = (140 - Age) \times \frac{Wt}{(72 \times Scr.)} \] for male

\[ Cl_{cr} = \left(140 - Age\right) \times \frac{Wt}{(72 \times Scr.)} \times 0.85 \] for female

Where Clcr is creatinine clearance in ml/min, age in year, Wt is the actual body weight or ideal body weight for obese patients (if BMI > 30) in kg, Scr is Serum creatinine in mg/dl.

- Css is the average steady state concentration of pregabalin was calculated based on the following equation:

\[ Css = \frac{AUC}{t_{1/2}} \]

**Objectives**

The objectives are Investigate the robustness of using a non-invasive saliva sampling method instead of a plasma sampling method as a surrogate for therapeutic drug monitoring of pregabalin among Jordanian patients.

**Experimental**

**Study designs and human subjects**

This study is an observational study that has been conducted at Islamic hospital/Amman. Forty four out patients with neuropathic pain, disc prolapsed, and diabetic neuropathy in neurology and orthopedic departments of Islamic hospital were enrolled in this study. The study includes forty four Patients with age ranging from 22 to 83 years (mean 48 years, ± SD 15). Female patients represent 38.6% while male patients represent 61.4% in this study. The actual weight of all patients is ranging from 62 to 145 kg (mean 89.9, ± SD 20.2). The ideal body weight was used for obese patients. The study was approved by Institutional Review Board (IRB) at Islamic hospital. The study was conducted under the supervision of Dr. Ahmad Alshalalfeh (consultant orthopedic surgeon-FACS- Islamic hospital). The criteria that should be available in the selected patients include: current therapy with pregabalin and patients should be at steady state concentration for pregabalin (steady state is achieved within 1 to 2 days of repeated administration), consent form is available.

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- Css is the average steady state concentration of pregabalin was calculated based on the following equation:

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Fraction absorbed (Fa) of pregabalin was calculated according to equations below:

\[ Fa = 1 - e^{-2An} \]  

\[ An = Peff \times tres/R \]  

Where An is the absorption number; Peff is effective intestinal permeability R and tres are radius, set at 1.75 cm, and mean residence time, set at 3 h, in the human small intestine respectively.

Optimized effective intestinal permeability

Effective intestinal permeability (P_{eff}) values were estimated by Nelder-Mead algorithm of Parameter Estimation module using PK-Sim program V7. Nelder-Mead method, which is also called downhill simplex, is a commonly used nonlinear optimization algorithm. This was done by searching for the best parameter values that produce plasma concentration that matches the actual plasma concentration at the same time. The objective function is the weighted sum of squared differences of observed and model predicted values.

All other physicochemical factors used in calculations such as Log P, MW and fu were obtained from literature and were kept constant during the minimization processes. In vitro dissolution rate, in vivo clearance and volume of distribution were input from actual dissolution and plasma profiles.

Statistical analysis

Microsoft Excel program was used for descriptive statistics and t-testing after log transformation, p-value = 0.05 for significant difference.

Individual data for each patient (age, gender, weight, height, dose, disease, r, serum creatinine, Cmin., Cmax and Css) and the appropriate equation for each pharmacokinetic parameter were entered to the excel program.

Results and Discussion

The average saliva pregabalin levels in the forty four patients involved in this study ranged from 4 to 331.19 ng/ml with a mean level of 55.85 ng/ml, Standard deviation = 55.46, CV % = 99.3 % as shown in Fig. 1. The steady state saliva pregabalin concentration was calculated according to eq. 3. In addition saliva/plasma ratio % ranged from 3.06% – 7.81 %, which indicated lower saliva drug levels.

The data clearly showed that there is no variability between trough saliva (Cmin) and maximum saliva pregabalin (Cmax) levels among patient as presented in Table 1, where a statistically significant difference (P < 0.90) which means that Tmax is not reached yet and so longer time is needed for Cmax (more than 1 h). From our proposed plasma pregabalin therapeutic range (0.7 to 1.84 µg/ml), we can find that ratio between Cmax/Cmin for plasma pregabalin is equal to 2.63 and so we can expect the Cmax for saliva pregabalin by multiplying the saliva pregabalin Cmax with 2.63. Based on the previous finding we suggest saliva pregabalin therapeutic range from 0.055 to 0.145 µg/ml.

The results showed that there is strong correlation found between Cmin of plasma pregabalin and Cmin of saliva pregabalin among patients; the correlation coefficient obtained is 0.71 as shown in Fig. 1. Also strong correlation found between Cmax of plasma pregabalin and Cmax of saliva pregabalin among patients, the correlation coefficient obtained is 0.83 as shown in Fig. 2.

Since there is a good correlation between Cmax and Cmin of plasma pregabalin and Cmax and Cmin of saliva pregabalin, this indicates that saliva sample can be used as an alternative matrix for pregabalin TDM.

Fig. 3 shows observed versus PK-Sim predicted pregabalin plasma concentration time profile. Optimized effective permeability coefficients was 9.9 × 10 − 3 cm/sec, with fraction absorbed Fa = 1.

Pregabalin as reported has high oral bioavailability ≥ 90 % (7). Based on the previous findings, that permeability and protein binding are major key factors in salivary excretion system [21], the result of this study showed that pregabalin has high permeability since high intestinal permeability corresponds to fraction absorption Fa > 0.9 and high protein binding corresponds to low fraction unbound fu of < 0.1 [22, 23]. Thus pregabalin is classified as class 1 drugs depending on SECS; drugs of high intestinal permeability and low protein binding [21]. Since pregabalin is class 1 drugs, it appears in saliva and this is consistent with our results.

And according to BCS (Biopharmaceutics Classification System) that had classified drugs based on their solubility and intestinal membrane permeability, Pregabalin is a BCS Class 1 compound (highly permeable and highly soluble). Pregabalin’s lowest aqueous solubility occurs at its isoelectric point (at pH 7.4). It is considered high soluble as the amount of water needed (< 10 ml) to dissolve the highest dose strength (300 mg) at pH 7.4 is less than the 250 ml criteria. Pregabalin meets the BCS criteria for a highly permeable compound as greater than 90 % of the dose is excreted unchanged in the urine [26].

The (plasma pregabalin concentration/dose) ratios were also calculated to demonstrate pharmacokinetic variability of Pregabalin. Factors that may contribute to pharmacokinetic variability of Pregabalin are gender, age (Patients ≥ 65 years were considered as elderly) and kidney function.

Moreover, the results showed that C/D-ratio in women (123.04) was higher than in men (10.57), also the ratio in elderly (21.59) was higher than non elderly (9.58) and the same thing regarding patients with Scr. ≥ 0.9 mg/dl who had higher ratio of 11.6 than patients with Scr. > 0.9 mg/dl who had a ratio of 9.79. The decrease in pregabalin clearance was compatible with increasing age (≥ 65 years) and reduced renal function (Scr. ≥ 0.9 mg/dl), and the higher ratio...
in women may be attributed to the difference in pregabalin renal clearance between males and females since normal range of creatinine clearance in women is (88–128 ml/min.) which is less than men (97-137 ml/min.).

These results are consistent with the reported studies related to pharmacokinetic variability of pregabalin which emphasize that pregabalin has extensive pharmacokinetic variability and that age and gender are contributing factors of this variability which elucidates the need for individualization of therapy and TDM [27, 28].

Table 1 Steady state trough and maximum plasma and saliva pregabalin levels (ng/ml) in 44 patients enrolled in this study.

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Future work

Longer time TDM study of pregabalin in saliva matrix in order to better catch actual $C_{max}^{SS}$ and $T_{max}^{SS}$.

Acknowledgments

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Conflict of Interest

The authors declare no conflict of interest.

References