THERAPEUTIC DRUG MONITORING OF VALPROIC ACID IN PEDIATRIC EPILEPTIC PATIENTS

Subash vijaya kumar¹, Y.Radhika¹, G.Vijayakumar², Ch.Ravikumar²

ABSTRACT

Therapeutic drug monitoring (TDM) or the measurement of drug concentrations in plasma, serum or blood, aims to improve clinical activity, avoid toxicity, and reduce the costs of drug treatment. Specific conditions for TDM to be reasonably applied include the availability of a validated assay, a considerable inter-individual pharmacokinetic variability, a high correlation between drug concentration and toxicity, and a narrow therapeutic index. Our aim of study is to investigate that, therapeutic drug monitoring of valproic acid in pediatric epileptic patients at tertiary care government hospital. The study was carried out prospectively during course of research activity in MGMH between March 2009 – October 2010. The study was approved by hospital ethical committee, Warangal. The patient group was selected from epileptic pediatrics patients who visited tertiary care hospital. All of the patients fulfilled the inclusion criteria and exclusion criteria. Informed consent was taken from the patients and guardian. Blood samples were collected from patients after morning dose at peak level. In our study total 30 pediatric epileptic patients are included in which 17 were males and 13 were females with mean age in years (7.93±3.38), mean BMI kg/m² (12.14±4.61) & mean total VPA serum concentration of 53.39±4.18 µg/ml . Serum levels within the therapeutic range were found in 46.33% of epileptic patient. Serum levels were below the therapeutic range in 56.66% of study population achieved partial control. Our article suggests that there was Poor correlation between daily dose and therapeutic level. There was a need for individual monitoring of liver function tests and blood urea nitrogen. In addition to that, a clinician has to check the therapeutic level or and despite daily dose being prescribed as per the guidelines. In future, clinician should step forward in the effort to ensure a more optimal and individual valproic acid therapy.

KEYWORDS

Therapeutic Drug Monitoring (TDM), Peak serum concentration, Valproic acid.

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INTRODUCTION

Therapeutic drug monitoring for valproic acid (VPA) which has the widest spectrum of activity among the available antiepileptic drugs. It is increasingly used for therapy of bipolar and schizoaffective disorders, neuropathic pain and for prophylactic treatment of migraine headache. Pharmacokinetics is highly variable; protein binding depends on concentration following therapeutic dose; and complex interactions between valproic acid and other drugs have been observed. The monitoring of valproic acid levels in the blood is necessary to determine appropriate dosage intervals and to assure proper therapeutic levels while avoiding hepatotoxicity.

Therapeutic and Toxic Ranges

Typical: 50-100 µg/mL / 347-693 µmol/L

Possible toxic levels: >100 µg/mL / >693 µmol/L

Valproate increases the levels of the inhibitory neurotransmitter, gamma amino butyric acid (GABA) at GABA-A and GABA-B receptors, inhibition of the catabolic enzymes succinic semialdehyde dehydrogenase and GABA transaminase. It has an elimination half-life of 9 - 21 hours (average 12 - 13 hours). It is metabolized principally in the liver and its metabolites are excreted in urine. Monotherapy possibly resulting from the activation of the synthetic enzyme glutamic acid decarboxylase and with valproic acid has demonstrated efficacy equivalent to that of carbamazepine, phenytoin, and phenobarbital in the treatment of both generalized and partial seizures and ethosuximide in the treatment of absence seizures. The dose is 20 mg/kg body weight this can be increased up to a maximum of 2500 mg per day in adults and up to maximum of 35mg/kg in children. Therefore our study is to investigate the therapeutic drug monitoring of valproic acid in pediatric epileptic patients at tertiary care government hospital.

MATERIALS

The study was carried out prospectively during the course of research activity in MGMH between March 2009 - October 2010. The study was approved by hospital ethical committee at Warangal. The patient group was selected from epileptic pediatric patients who visited tertiary care hospital. All of the patients fulfilled the inclusion criteria (Epilepsy patients using Valproic Acid Monotherapy and Age 2 – 14yrs., either Sex) and exclusion criteria (Severe disability, Pregnancy and Lactation, Elderly any other disease, Under Poly pharmacy). Informed consent was taken from the patients and guardians. All the patients relevant information were collected like name, age, sex, body weight, type of seizures, biochemical & electro physiological investigations. [Hb, CBC, BUN, SGPT, SGOT] EEG, duration of seizures, family history, present medication, co-medication, starting dose, side effects date & time of last dose taken & sampling time. Blood samples were collected from patients after morning dose at peak level. The collected samples were stored at -20°C until further analysis was done. Samples were measured by a specific and sensitive high performance liquid chromatography method (Kishore et al., 2003). After analysis of the blood sample on HPLC, the serum drug level of the sample taken from patient was reported. The patients biochemical parameters was compared with 56 healthy children from government high school, Kazipet, Warangal, A.P. Statistical analysis was performed by using SPSS software. Data was expressed as mean ± SD. Levene’s test was used for comparison of two groups. A ‘p’ value less than 0.05 was considered as statistically significant.
METHODS

Procedure for Preparation of Standard Solutions

Stock solutions of 1 mg/ml of VPA and 0.01 mg/ml of diazepam were prepared in methanol and acetonitrile respectively and stored at –4°C. VPA stock solution was further diluted with methanol to the required concentration (20, 50, 80, 120 and 150 µg/ml). Calibration samples were prepared by spiking 250 µl blank serum with appropriate amount of drug on the day of analysis. Sample for the determination of recovery, precision and accuracy were prepared by spiking control human serum in bulk of appropriate concentrations (20, 50, 80, 120 and 150 µg/ml) and stored at -20°C.

Extraction Procedure

To 250µl serum samples, acetonitrile solution of diazepam equivalent to 2.5 µg was added as I.S and shaken well. Then equivalent amount of (250µl) acetonitrile was added for protein precipitation and mixed on a cyclomixer for 1 minute and centrifuged at 4000 rpm using a REMI centrifuge for 20 min. 50 µl of the supernatant was injected on to HPLC column.

RESULTS

A total 30 subjects where include the study, the mean age of the patients 7.93 ±3.38 and male were predominant in this group, the value of SGOT, SGPT, BUN, Serum amylase, Hemoglobin and sodium valproate were shown in table no: 1

Chromatography

Chromatogram of valproic acid and internal standard diazepam has been shown in figure no.1

![Chromatogram](image.png)

Fig.1 Chromatogram of Standard valproic acid and internal standard diazepam

<table>
<thead>
<tr>
<th>Drug</th>
<th>Rt</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valproic acid</td>
<td>2.670</td>
<td>40380</td>
</tr>
<tr>
<td>Diazepam</td>
<td>8.063</td>
<td>806741</td>
</tr>
</tbody>
</table>
and it shows that there was no interfering peak observed when the sample chromatogram was analyzed in figure no. 2

![HPLC Chromatogram of Sample valproic acid and diazepam](image)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Retention time</th>
<th>Area</th>
</tr>
</thead>
<tbody>
<tr>
<td>Valproic acid</td>
<td>2.686</td>
<td>20152</td>
</tr>
<tr>
<td>Diazepam</td>
<td>7.634</td>
<td>836368</td>
</tr>
</tbody>
</table>

Plot the peak height ratio between sodium valproate and diazepam Vs concentration of the drug to construct the calibration curve using the result from serum standard and serum blank. Drug concentration in the patient serum can be calculated by this method.

**Blood Sampling and Drug Analysis**

Peak Serum samples were taken after the administration of the morning dose. High performance liquid chromatography (HPLC) method was used for determination of the serum VPA concentration. VPA serum sample was collected after 3 hours of the administration in heparinized vial and were either assayed immediately or frozen for assay at later date.

**Sodium Valporate Assay**

A serum sample from 30 patients were screened from the present of internal standard, diazepam using a HPLC assay serum (0.5-1ml), applied to a C18 column. Mobile phase consisting of acetonitrile and 0.05 M potassium dihydrogen ortho phosphate (pH adjusted to 3 with ortho phosphoric acid) (45:55 v/v) was used at a flow rate of 1.2 ml/min. The eluate
was monitored at dual wavelength of UV detector at 210nm from 0 to 10min, retention time for sodium valproate and diazepam was 2.6 to 7.63 min respectively. The sensitivity was set at 0.001 A.U.F.S. This assay can detect the concentration of VPA. Retention time of patient serum sample and pure drug (Valproic acid) serum sample along with internal standard (diazepam) are almost correlating with each other.

**Characteristics of the Study Population**

The study population consisted of 30 pediatric’s epileptic patients. Demographic and medication details for the patients were summarized in Table 1.

**Table 1: Bio-Parameter Study of Various Group Population in Numerous Pediatric Patients**

<table>
<thead>
<tr>
<th>Patients (n)</th>
<th>Samples(n)</th>
<th>Controls (n) 56</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>17 (56.66%)</td>
<td>26 (46.42)</td>
</tr>
<tr>
<td>Female</td>
<td>13 (43.33%)</td>
<td>30 (53.53)</td>
</tr>
<tr>
<td>Mean Age (yrs)</td>
<td>7.93 ± 3.38</td>
<td>8.75 ± 2.84</td>
</tr>
<tr>
<td>Mean Total body weight (kg)</td>
<td>19.20 ± 8.95</td>
<td>20.96 ± 6.92</td>
</tr>
<tr>
<td>Mean BMI</td>
<td>12.14 ± 4.61</td>
<td>10.32 ± 3.26</td>
</tr>
<tr>
<td>Serum VPA concentration (µg/ml)</td>
<td>53.39 ± 4.18</td>
<td>-</td>
</tr>
<tr>
<td>Mean SGPT</td>
<td>18.29 ± 19.20</td>
<td>24.36 ± 5.92</td>
</tr>
<tr>
<td>Mean SGOT</td>
<td>28.50 ± 31.44</td>
<td>27.96 ± 8.97</td>
</tr>
<tr>
<td>Mean BUN</td>
<td>28.15 ± 20.78</td>
<td>30.01 ± 9.15</td>
</tr>
<tr>
<td>Mean Serum amylase</td>
<td>28.01 ± 19.58</td>
<td>39.52 ± 7.81</td>
</tr>
<tr>
<td>Mean Hemoglobin</td>
<td>10.87 ± 2.07</td>
<td>10.56 ± 0.62</td>
</tr>
</tbody>
</table>

n=30; where n is the number of patients used for the study

All of the patients were on monotherapy.

From the above mentioned table patients were 30 and controls were 56 baseline characteristics of all parameters are observed and each parameter was explained in discussion, whereas, descriptive analysis was carried out by SPSS software for windows version17, USA.

**TDM of VPA**

The high portion of pediatric epileptic patients in whom VPA level was in sub therapeutic range (53.33%) showed the partial response and (46.66%) of patients who had a VPA level in therapeutic range didn’t show complete response.
Table 2: TDM of valproic acid and its therapeutic range.

<table>
<thead>
<tr>
<th>Therapeutic range</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Within the range</td>
<td>46.66</td>
</tr>
<tr>
<td>Sub-therapeutic range</td>
<td>53.33</td>
</tr>
<tr>
<td>Toxic level</td>
<td>0</td>
</tr>
</tbody>
</table>

**DISCUSSION**

Our results indicate that total 30 pediatric epileptic patients were on long term valproic acid oral therapy for at least six months. The daily oral medication (tablet or syrup) was given in two divided doses (minimum dose 15mg/kg/day). The children age were ranged from 2-14 years. Among them males were 17 (56.66%) and females were 13 (43.33%). The patients with known family history were 5 (16.66%) and with no significant family history were 25 (83.33%). Table 1 shows range mean± SD values of age, sex, body weight, SGPT, SGOT, BUN, Serum amylase, Hemoglobin and VPA. Among 30 epileptic patients in 17 (53.33%) valproic acid levels were in sub-therapeutic range and in 13 (46.66%) with in therapeutic range these patients had partial seizure control. This study was in contrast Irani population (Mohsen et al 2009)11. Other studies had similar observations that valproic acid with in range 40% had recurrent attacks (as noted by Kutte et al 1964; kuttanol P 1974; Morselli 197713). Clinical effects are more closely related to drug levels than to the dose TDM is particularly useful in determination of drug levels and identification of therapeutic failure due to under dosage and even at in presence of optimal dosage for identification serious toxicity inter individual pharmacovariability. (Sharma S et al 2008)13. Though SGPT mean is statistically not significant but SGPT levels were elevated individually in 7 patients. (23%) Though SGOT mean is statistically not significant but SGOT levels were above the upper limit in 8 individual patients (26%). (Similar observation was made by David LC et al 1980)14. The overall BUN mean is statistically not significant (P value 0.05) but in 15 children BUN is elevated in 9 patients and in 6 controls. Here mean of elevated BUN in patients was 56.16±15.182 and mean of elevated BUN in controls was 42.57±2.620. ’t’ test of unequal variances were 2.8 and df was 8.706. ’t’ tailed ‘P’ value was 0.02 which was highly significant in patients. The elevated BUN levels may be due to elevated serum ammonia levels and diet. But patients do not have any other disease presumably both patients and controls were from same group.

Serum amylase levels should be measured in children receiving valproic acid with recurrent vomiting and abdominal pain to detect valproic acid associated pancreatitis (David LC et al1980). The mean serum amylase in patients (28.01±19.58) and in controls (30.01 ± 27.84) was statistically not significant but biologically it had significant value as identified by outliers of Masuyama’s rejection limit test. Frequency of side effects was similar to other observations (i.e., no pancreatitis and thrombocytopenia in our series. We found poor correlation (r²=0.01) between daily dose and therapeutic level of drug. This may be because of poor compliance or inter individual pharmacokinetic variability (rapid and slow metabolism of the drug. But above aspects were not our objective of the present study. Similar observations were noted (May and Rambeck 198515), (Mesdijian et al.198416). A weak correlation was found between valproic acid concentration and dose. Only few authors found a high linear correlation between the valproic acid and concentration (Klotz 1977) or yet a clearly non linear dose/level relationship (Vajda et al 1978). The relationship between the valproic acid dose and concentration is not linear (May T and Rambeck 1985).15
Excessive weight was observed in total of one patient in our study. As mentioned in literature, weight gain was seen at therapeutic range. Weight gain occurs mainly during the first 6 months of valproic acid treatment and then continuous at a lower rate even if the valproic acid dose is increased. This independence of dose has been reported previously. Most of the patients has GTCS in 9 (30%) total seizure control with valproic acid treatment of epilepsy was reported to be achieved at 55% to 100% (Covallis et al 1982). As suggested by Gram (Gram et al 1979), lower limit of 300 to 350µmol/L was associated with better seizure control.

CONCLUSION

Our article suggests that, there was poor correlation between daily dose and therapeutic level. Whereas, need for individual monitoring of liver function tests and Blood urea nitrogen is needed. Moreover, there was no significant difference between the mean serum levels with seizure control. Pediatric patients on mono therapy effective concentration of valproic acid varied from 16.55 to 84.20µg/ml. In addition to that, a Clinician has to check the therapeutic level on and despite daily dose being prescribed as per the guidelines. We hereby conclude that the clinical utilization of our findings might be a step forward in the effort to ensure a more optimal and individual valproic acid therapy.

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