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Biochemical Monitoring of Antiepileptic Drugs: A Comparative Assessment of Enzyme Multiplied Immunoassay, Spectrophotometric and Colorimetric Techniques

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Abstract

A comparative assessment was made of three different methods used in the assay of AED in body fluids. These included the Colorimetric, Spectrophotometric and Enzyme Immunochemical techniques. The merits and demerits are discussed. The ideal technique would be EMIT in situations where quick reporting is needed.

Key words -

Antiepileptic drugs,
Emit,
Colorimetry,
Spectrophotometry

Epileptic patients constitute a sizeable number of population in any neurological clinic including NIMHANS. The effective clinical management of epileptic patients is by using antiepileptic drugs (AED) [1]. It is ideal to monitor the AED level in blood during the drug regimen [1], [2]. Therapeutic drug monitoring (TDM) of AED, greatly helps in proper adjustment of dosage to give adequate benefit to the patients [2]. Increased application of TDM has been facilitated due to the development of rapid, specific and reliable assays of AED in body fluids [3], [4], [5].

The paper reports experiences in the use of enzyme immunochemical technique based on homogenous assay procedure for monitoring Phenytoin (DPH) and Phenobarbitone (PB) as well as its comparison with two other techniques for assaying AED.

Material and Methods

Samples of blood collected from epileptic patients who were on drugs, formed the material for this study. The drug levels in blood samples were assayed by enzyme multiplied immunoassay technique [3] (EMIT - Syva^{R}) using Gilford Spectrophotometer- Stasar III, System 4.

The same blood samples were processed simultaneously for drug assay by using two other methods viz., Colrimetry [6] and Spectrophotometry [7] in the UV range. Both these methods required prior extraction of the drugs. In the Colorimetric method the extracted drug (Phenytoin) was converted to a coloured derivative and the resulting purple colour read in a Klett-Summerson Colorimeter at 540 nm. In the Spectrophotometric method PB was separated from DPH during the extraction procedure. PB was quantitated by ultraviolet absorption (at 240 nm) at pH 8.8 and 1.5 while DPH was estimated by noting the absorbance change at 235 nm and 260 nm (at pH 11.5) using UV/VIS Beckman Spectrophotometer.

Results and Discussion

From the values obtained by analysis of 75 blood samples simultaneously for DPH and PB estimations, it was noted that the optimum (effective) therapeutic concentration ranged from 10-20 micrograms/ml for DPH and from 15-50 micrograms/ml for PB. All the samples were analysed for the two drugs by all the three methods. The results obtained by each of these method were compared, and it was noted that at lower concentrations i.e., below 8 micrograms/ml for DPH, there was some correlation between the values obtained by the Colorimetric method and Emit. However this is apparent and not real, since it has been known that the Bratten-Marshal reagent used, gives a value upto 5 micrograms/ml even in normal serum [8] (commonly referred to as the Dill positive substances). Thus the Colorimetric method has an inherent error. This is also supported by the fact that at concentrations above this level, the Colorimetric method shows values 15-30% above the ones obtained by using Emit [9], [10].

Different parameters, such as sample volume, specificity, interference and rapidity of method, were compared in the three methods used for AED monitoring viz., Emit, UV-Spectrophotometry and Colorimetry. It was noted that Emit requires only 50 microlitres (0.05ml) of sample where as for Colorimetric and UV methods, a minimum of 1 ml sample is required. It is not possible to make satisfactory extraction below this sample volume. Since the entire procedure of Emit is so well adjusted, to take very small volumes of 0.05 ml, as to enable samples from children to be analysed (where collection of large volumes of blood is somewhat of a problem). The accuracy of Colorimetry and UV methods actually increases with volumes above 1ml.

Emit is highly specific for AED assay [3] and there is no interference by other compounds unlike in the other two methods [11].Since the Emit is based on the principle of antigen antibody reaction involving specific antibodies raised against the drugs, this avoids any interference by other compounds present in the serum. Whereas in the other two methods not only the other drugs and their metabolites present, but also many endogenous compounds structurally resembling the AED, are measured giving higher values [9], [10]. Thus Emit is highly specific for AED assay. Due to the high degree of specificity, Emit assay becomes particularly useful when the AED levels have to be estimated in epileptic patients who are on multiple drugs, (as well as sometimes on drugs other than AED) and when there is every chance of variation of AED levels due to drug interaction [10].

Since Emit assay can be completed in less than a minute, the time required to complete one assay by each of the 3 methods was then compared. It was seen that Emit is the most rapid method (less than 60 secs for a single assay) as compared to UV and Colorimetric methods which takes hours. Moreover in the Emit procedure, the extraction of AED from serum samples is not necessary and since it is a

homogenous enzyme immunoassay (EIA) there is no need to separate bound and free form of enzyme labelled drugs unlike other heterogeneous forms of EIA, eg. ELISA, thus reducing the assay time to a great extent and providing the rapidity and quickness to the technique whereas the time required for single assay by UV Spectrophotometry is much more (about two hours) than Emit because it requires extraction of the drugs from the samples prior to their quantitation. Colorimetry takes almost four hours since in addition to the tedious multistep extraction of AED from the samples (like UV method) the preparation of color derivatives also require considerable time, thus increasing the assay time.

In cases of accidental drug intake it is possible to get the report within the shortest time using Emit, so much so the data is of immediate relevance for management purposes. In such of those individuals who were not epileptics but who took these medicines in large doses, we found [10] that the assay by Emit was of great practical importance as the results were obtained in less than 60 seconds. This method was also found to be most useful in cases where despite adequate dosage, the patient came back with uncontrolled seizures. They invariably showed subtherapeutic levels [10], [11] (probably indicating irregularity of drug intake or intake of low doses with a sense of apparent well being).

It would not be out of place to indicate that the method of choice used by many centres abroad is Gas Chromatography (GC). A study carried out by Pippenger et al [12] showed that there is wide interlaboratory variability in determination of AED levels which is unacceptable. Another study conducted by Spiehler et al [5] for interpretation of clinical status of the patient compared several methods of AED determinations including GC and Emit. They concluded that Emit can be substituted for Liquid Chromatography or UV Spectrophotometry without changing the resulting clinical interpretation. A study carried out by Stanley et al [4] showed that for AED assay, the GC method is inexpensive in so far as reagents are concerned but otherwise it is costly in instrument requirement. An analysis of cost benefit aspects showed that of the three methods, Emit is the most expensive method [10]. But in view of the benefits indicated, it could be a method of choice for TDM. It is to be noted that NIMHANS is the only centre in India, at present which is using Emit, which is a non-isotopic homogeneous enzyme immunochemical assay for AED monitoring.

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