ASSESSMENT OF A NEW ULTRAVIOLET SPECTROPHOTOMETRIC METHOD FOR DETERMINATION OF THEOPHYLLINE IN PLASMA

Routine monitoring of plasma theophylline levels as an aid to therapy is becoming increasingly popular, and a variety of methods exist for measurement. The criteria for a good assay include: 1) small sample size, which facilitates measurement of plasma theophylline levels in children and infants, 2) speed of assay, 3) low overall cost and 4) good reproducibility. In our laboratory, we have been measuring plasma theophylline levels for 2 years, and have examined the feasibility of several methods of analysis. Recently, we assessed the performance of a new ultraviolet spectrophotometric (UV) assay for theophylline (Hohnadel, Grove & Alonzo, 1978), which offered the advantages of small sample volume and the use of relatively low-cost and readily-available equipment. We report here the results of a comparison between this method and two established techniques in routine use in our laboratory, a gas-liquid chromatography (GLC) assay (Dusci, Hackett & McDonald, 1975) and enzyme immunoassay (EMIT: Syva, England).

Fifty samples of patient plasma, arriving in our laboratory for routine analysis, were arbitrarily selected and assayed by each of the three methods. Samples were stored frozen at -20°C until assay. The UV and EMIT techniques required only $50\mu\text{l}$ of plasma, whereas GLC required 1 ml for assay. None of the three methods was affected by the presence of caffeine, but theobromine interfered with the UV assay (Hohnadel *et al.*, 1978). Patient plasma used in this study was not checked for the presence of these substances. Results were recorded, and the different methods compared using regression analysis by the method of least squares. Figure 1 shows the graphs obtained.

GLC and EMIT (Figure 1a) showed approximately equivalent quantitation, with the experimentally-derived regression line lying close to the line of identity. There was a slight tendency towards higher results with GLC, the y-intercept lying at approximately $7\mu\text{mol l}^{-1}$.

UV and EMIT (Figure 1b) showed a slightly poorer correlation, but a consistent trend for UV to give higher levels than EMIT was the most obvious feature. In some cases this was quite marked, the value obtained from the UV assay being almost twice that from EMIT. In particular, two samples shown by EMIT and GLC to have negligible theophylline present were shown by UV to have significant concentrations, in one instance almost within the therapeutic range of $56\text{--}112\mu\text{mol l}^{-1}$.

In Figure 1c, it can be seen that in our study, GLC

and UV correlated extremely poorly. There was a considerable degree of scatter in the values obtained using each method, and again a tendency for UV to give high readings.

In assessing these results, the characteristics of each of the three assays must be considered. The EMIT enzyme immunoassay, of the three, is the most specific. Caffeine and theobromine in normal amounts in plasma do not cross-react with theophylline, nor do its principal metabolites. However, as this assay involves the use of a spectrophotometer to make readings on diluted but unextracted samples, haemolyzed samples may produce spurious results, and a similar degree of variability would be expected to occur with lipaemic specimens. Such samples, therefore, should be analyzed using a different technique. None of the patient samples in this study was grossly haemolyzed or lipaemic but slight variations in these factors could cause some of the scatter observed in the results.

The GLC method used is also specific for theophylline, and a double extraction procedure removes caffeine from the samples. This is particularly important with this assay as it depends on methylation of theophylline to caffeine prior to analysis. However, a disadvantage of this method is that the internal standard, medazepam, is not added until after the extraction is complete, and it therefore cannot be used as a control to compensate for losses of sample during extraction. In spite of this, we have found this technique to be reproducible, with a coefficient of variation of 10–15%, compared to 5–10% for EMIT. High blank values may occasionally occur when patients are receiving other drugs in addition to theophylline.

In our hands, the UV assay of Hohnadel *et al.* (1978) has shown several disadvantages over EMIT and GLC procedures already in use in our laboratory. Compared to both EMIT and GLC, UV gave consistently high results, in spite of strict adherence to the protocol described for each assay. Hohnadel *et al.* (1978) reported a rather better correlation between EMIT and UV than was found in the present study, but their results also show a tendency for UV to give higher results. Good agreement with a high-pressure liquid chromatography method was observed, but this result was obtained on only one half of the number of samples used for comparison with EMIT. It is possible that this UV assay may also measure a metabolite of theophylline, and this would cause it to give higher

Figure 1 Regression analysis of plasma theophylline levels a) GLC v EMIT, b) UV v EMIT, c) UV v GLC.

readings with patient samples. This would not be so if plasma samples were spiked with theophylline *in vitro*, and much better agreement between methods would then be expected. However, this aspect was not examined in this study. As mentioned previously, samples were not checked for theobromine content, and this substance interferes with the UV assay.

References

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However, since it would not be possible in the situation of routine blood sampling to control the diet of patients on theophylline therapy, any assay which would be affected to this extent by interfering substances could not be regarded as acceptable.

Our study has shown that in our laboratory two established methods for analysis of theophylline in plasma, GLC and EMIT, appear to give comparable results, with an acceptable degree of variation in most cases, variability probably being slightly greater with GLC because of the inherent characteristics of the particular assay used. However, the UV assay of Hohnadel *et al.* (1978) did not correlate well with either method, its principal fault being a trend towards high readings. We therefore do not recommend the use of this method in routine clinical laboratories for assay of plasma theophylline until the cause of this problem has been resolved, as inaccuracies may occur in estimation.

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