Metabolism of didanosine (ddI) by erythrocytes: pharmacokinetic implications

Didanosine is a nucleoside analogue with potent inhibitory effects against the human immunodeficiency virus (HIV) [1]. It is converted within target cells to its active form ddA-triphosphate, which is thought to act as a chain terminator and inhibitor of reverse transcriptase [2]. In addition to the intracellular formation of ddA-TP, didanosine may be broken down to hypoxanthine and uric acid by the enzymes purine nucleoside phosphorylase (PNP) and xanthine oxidase [3]. As PNP is found in relatively high concentrations in human erythrocytes [4] we have previously investigated the metabolism of ddI *in vitro* using human blood [5]. When incubated with whole blood at 37° C, ddI was extensively metabolised, principally to hypoxanthine (50% formed at 6 h). Metabolism to hypoxanthine occurred within red blood cells and was temperature dependent. Following this *in vitro* study we suggested that the metabolic degradation of ddI within red blood cells may have implications for the interpretation of the results of ddI pharmacokinetic studies.

We have now investigated this possibility in a pharmacokinetic study of three men who were infected with HIV and who took ddI (250 mg twice daily) following zidovudine intolerance. Approval for the study was granted by the local Ethics Committee and all patients provided written informed consent. Each patient attended for study on two occasions separated by at least 2 weeks. After an overnight fast and following the insertion of an intravenous cannula, blood samples were taken at 15 min intervals for 2 h, half-hourly for another 2 h, and at 5 and 6 h after didanosine 250 mg.

Each blood sample was divided into two aliquots, one was centrifuged immediately (3000 rev min^{-1} for 10 min) and the other was left at room temperature and centrifuged when the pharmacokinetic study was completed. The separated plasma samples were exposed to a temperature of 58° C for 30 min to inactivate the human immunodeficiency virus [6] and then analysed for ddI using a commercial radioimminoassay (Sigma, London). Plasma samples were initially diluted 1:100 with blank plasma. The assay has a limit of detection of less than 1 nM. The interassay coefficient of variation was less than 10% at a concentration of 10 nM. Figure 1 shows the plasma drug concentrations for samples centrifuged immediately and those centrifuged after some delay, i.e. at the end of the study period. Although the latter were left for variable times (early samples left longer than later samples) this procedure reflects what will often happen in clinical studies.

Plasma concentrations of ddI were consistently lower in samples whose centrifugation was delayed, with the greatest difference in early samples and little difference in later samples. The area under the curve (AUC(0,6h), calculated by the linear trapezoidal rule, was reduced by

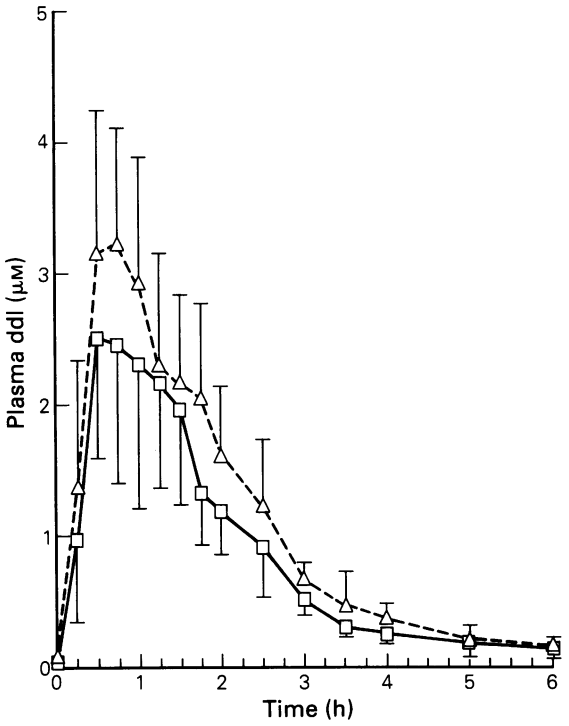

Figure 1 Mean (± s.d.) plasma concentrations of ddI (μM) following a single oral dose (250 mg). Blood samples were centrifuged immediately after collection (no delay, ▲) or left at room temperature until the end of the study prior to centrifugation (delay, □).

22% from 6.69 ± 1.12 to 5.21 ± 0.86 μmol l^{-1} h (mean ± s.d; $P < 0.001$, Student's paired *t*-test; 95% CI of the difference 1.00 to 1.95). The maximum plasma drug concentration (C_{max}) was reduced from 3.86 ± 0.66 to 3.10 ± 0.77 μmol l^{-1} ($P < 0.01$; 95% CI of the difference 0.22 to 1.30).

The implications for pharmacokinetic studies involving ddI are clear. If blood samples are taken and left at room temperature (or higher) for any length of time, concentrations of ddI will be underestimated because of its continued breakdown in blood. Therefore, blood samples should be centrifuged immediately and the plasma separated to minimise breakdown by PNP.

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Impedance cardiography

We read the article by Thomas on impedance cardiography (TEB) [1].

The author rightly commented that statistical methods used by previous authors in assessing the accuracy of the Sramek-Bernstein method were inappropriate. However, we also question the way in which the method of agreement [2] was applied in this article.

According to the method suggested by Bland & Altman [2], certain criteria should be fulfilled before the limits of agreement can be calculated. These are:

1. Data points must be independent of each other.
2. The data must have a normal or near normal distribution.
3. There must be no systematic bias between the two methods (i.e. mean difference not significantly different from zero at the 5% level).
4. The 95% limits of agreement is (bias ± 2 s.d.) only if the number of subjects being studied provide a t value of approximately 2.0 at $P = 0.05$.

In the article by Thomas [1] the limits of agreement between TEB and other methods were all calculated using multiple data points from each subject. The data points are therefore not independent. In the comparative study between TEB and dye dilution, only five subjects were studied. It would be difficult to ascertain normal distribution of the data in so few subjects. Also, with only five subjects, the 95% limits of agreement would not be (bias ± 2. s.d.), but should be almost (bias ± 3 s.d.). Since there was systematic bias between the changes measured by each method, it is inappropriate to calculate the limits of agreement by any means.

Similarly, for linear regression and correlation analyses, independent data points should be used. Otherwise, the relationship can be artificially improved by within-subjects correlation. However, as the data of the study comparing TEB with oxygen consumption were not shown, we are uncertain how the correlation was calculated.

TEB has great potential in clinical pharmacological studies in normal subjects [3], but it is not accepted for clinical use because of doubts about its accuracy. If the full potential of the TEB is to be achieved, comparative studies similar to those of Thomas [1] are essential, but their statistical analysis must be more rigorous if they are to achieve their aims.

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