Deacon's Challenge No. 88 Answer

Drug A is routinely used in the treatment of patients with rheumatoid arthritis. It is metabolized *in vivo* to its active metabolite B by the enzyme PP. The possibility of introducing drug C into the treatment regimen is being investigated but there are some concerns that drug C may inhibit the metabolism of drug A. In order to investigate the effect of drug C on the metabolism of drug A the effect of varying the concentration of drug A on the activity of the enzyme PP was investigated in both the presence and absence of drug C. The method for measuring the activity of PP is:

0.5 mL substrate (drug A)
1 mL Reagent 1 (contains the enzyme PP)
2 mL reagent 2 (contains a second enzyme which converts B into a coloured end-product)
0.5 mL of buffer OR buffer containing drug C at a concentration of 50 mmol/L

The rate of formation of the coloured end-product was measured by following the increase in absorption at 505 nm.

The double reciprocal plots (1/[S] versus 1/v) were linear. In the absence of drug C the Km of the enzyme was found to be 80 µmol/L and the Vmax 200 µmol/min/L. In the presence of inhibitor the apparent Km was 280 µmol/L with an apparent Vmax of 195 µmol/min/L.

a) What is the most likely mode of inhibition?
b) Calculate the inhibitor constant.

Based on MRCPath practical, Autumn 2003

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a) Since the addition of inhibitor increases the Km (i.e. lowers the affinity of the enzyme for its substrate) without any significant effect on Vmax the most likely mode of inhibition is competitive.

b) The effect of a competitive inhibitor on the Km of an enzyme is described by the expression:

Apparent Km = Km (1 + [I]/Ki)
Apparent Km = Km measured in presence of inhibitor = 280 µmol/L

Ki = inhibitor constant (i.e. dissociation constant of the enzyme- inhibitor complex)
[I] = inhibitor concentration

Since 0.5 mL of the inhibitor solution (drug C at a concentration of 50 mmol/L) is diluted to 4.0 mL for the assay, the concentration of the inhibitor in the reaction mixture is 50/8 = 6.25 mmol/L.

Therefore substitute [I] = 6.25 mmol/L, Km = 80 µmol/L and apparent Km = 280 µmol/L then solve for Ki:

$$280 = 80\left(1 + \frac{6.25}{Ki}\right)$$

$$\frac{280}{80} = 1 + \frac{6.25}{Ki}$$

$$3.5 = 1 + \frac{6.25}{Ki}$$

$$3.5 - 1 = \frac{6.25}{Ki}$$

$$2.5 = \frac{6.25}{Ki}$$

$$Ki = \frac{6.25}{25} = \mathbf{2.5\ mmol/L}$$

Question 89

An adult male (body weight 82 Kg) produces a 24h urine collection with a total volume of 1.56 L and a creatinine concentration of 9.5 mmol/L. His plasma creatinine concentration (in a blood collected during the urine collection period) was 95 µmol/L. Estimate the half life of plasma creatinine stating any assumptions that you make.