DETERMINATION OF METHOTREXATE SERUM CONCENTRATIONS FOLLOWING HIGH DOSE INFUSION THERAPY

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High dose methotrexate infusion therapy with folinic acid rescue is now being used in the treatment of some solid tumours in man previously resistant to conventional drug therapy (Isacoff et al 1976). It is thought that the higher blood concentration achieved with high dose methotrexate administration will generate higher intracellular drug concentrations and hence greater anti-tumour effect (Djerassi 1975). However, high blood levels of methotrexate if maintained for prolonged periods can result in the development of severe toxicity (Friedman & Carter 1978). Thus, a need exists to monitor blood methotrexate levels following this dosage regime to indicate whether a patient is able to rapidly eliminate the drug, or whether toxicity may arise as a result of maintained blood levels.

We have developed a relatively simple enzyme-inhibition assay for this purpose based upon the method of Falk et al (1976). Samples containing methotrexate were pre-incubated with an enzyme-NADPH mixture in sodium phosphate buffer (pH 7.4) and the reaction started by the addition of the substrate, dihydrofolic acid. This method was then compared and contrasted with a commercially available immunoassay procedure (Syva, Maidenhead). Good correlation was obtained between the two methods of assay ($r = 0.993$; $n = 120$) and there were no significant differences using serum, plasma or urine samples. Both methods are rapid to perform and have the same degree of accuracy and reliability, however the enzyme-inhibition is more sensitive and can be carried out at a fraction of the cost of the commercially available system.

In a patient study, ten patients with advanced malignant disease received 1g/24 h infusion of methotrexate and serum concentrations were determined for periods up to 250 h. On completion of the infusion, methotrexate serum levels averaged $3.1 \times 10^{-5} \pm 0.41 \times 10^{-5}$M being generally eliminated over the next 100 h. Elimination followed a triphasic pattern, the half-lives after a single infusion ranging from 2.7 - 4.5 h for the first elimination phase, 5.1 - 14.4 h for the second and 8.1 - 23.5 h for the third. Patients later received up to five further infusions of the same dose of methotrexate at intervals of 3 weeks. In nine patients, elimination half-lives for each elimination phase were of the same order for each successive infusion. However, in one patient, half-lives were significantly increased on successive infusions indicating a reduced ability of this patient to excrete methotrexate. This information allowed folinic acid rescue to be continued for longer than normal thus avoiding impending toxicity.

The results of this study indicate that the enzyme inhibition assay provides a sensitive, reliable and practical method of determining methotrexate concentrations in biological fluids. The determination of elimination half-lives in patients treated with this drug enables the drug to be used with greater safety.

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