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SHORT COMMUNICATION

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Interference by antifolate drugs in a folate competitive protein-binding assay

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Thymidylate synthase (TS, EC 2.1.1.45) and other folate-dependent enzymes have recently received much interest as chemotherapeutic targets and several compounds are in preclinical and clinical study [5]. One of these, ZD1694 (Tomudex), a polyglutamatable quinazoline antifolate TS inhibitor [6], has recently been registered in the United Kingdom for the treatment of advanced colorectal cancer following phase III trials [3]. During a phase I trial of this compound [2], plasma and red blood cell (RBC) folate levels were measured using a competitive protein-binding (CPB) assay (Becton Dickinson UK Ltd., Cowley Oxford) prior to each course of treatment. Before the first course of treatment, folate levels were within the range of expected values, but these were frequently elevated (~2-fold) following the administration of ZD1694. Pharmacokinetics studies in patients [2, 8] have shown that ZD1694 has a long terminal elimination phase and that drug is present in the plasma for prolonged periods after administration. There was also evidence of some drug accumulation in RBCs. In view of the known cross-reactivity of methotrexate in CPB folate assays (Product Information, Becton Dickinson; Kodak Clinical Diagnostics Ltd.; [9]) we evaluated the effect of ZD1694 and other antifolate drugs on the measurement of plasma folates using this kit. The assay was carried out as described in the instructions that accompanied the kit and the results were compared with those obtained with a radioimmunoassay.

The radioimmunoassay used an antibody to 5-methyltetrahydrofolate (CH_3-THFA) that had been raised in a sheep to an ovalbumin-CH_3-THFA immunogen, and because of the low specific activity the radiolabel ([5-C^{14}]-CH_3-THFA barium salt, 55 mCi/mmol; Amersham International plc, CFA371) was used at a dilution of only ⅕. However, this was sufficient to produce a standard curve for CH_3-THFA covering the concentration range of 3–300 ng/ml. This compares with a standard range in the CPB assay of 1–20 ng/ml.

Several antifolates were used in both assays to assess the specificity of binding. In the CPB assay the quinazoline antifolates ZD1694 and ZD9331 [7], the glycinamide ribonucleosyl (GAR) transformylase inhibitor lometrexol [1] and the TS inhibitor BW1843U89 [4] cross-reacted as least as well as CH_3-THFA for the milk-binding protein in the kit. The TS inhibitors LY231514 [11] and AG337 [10] cross-reacted by 50% and 2%, respectively. Methotrexate cross-reacted by only 2.5%. In contrast, none of these compounds was recognised by the CH_3-THFA antiserum (percentage of cross-reaction $<<1\%$).

For determination of the extent to which antifolates might interfere with folate measurements, normal plasma was spiked with ZD1694 to give final concentrations of 1, 10 and 100 ng/ml. Table 1 shows the results obtained by both CPB assay and radioimmunoassay.

In summary, a number of antifolates have been shown to have significant "cross-reactivity" in the CPB folate assay kit. This is consistent with published work showing that a wide range of antifolates bind (with differing affinities) to the membrane-associated folate transport protein, the mFBP [12]. In contrast, the antibody to CH_3-THFA used in a radioimmunoassay is highly specific and does not cross-react with several natural folates, including folinic acid (0.1%), tetrahydrofolic acid (0.1%), dihydrofolic acid

Table 1 Results of the CPB assay and the radioimmunoassay

Added ZD1694 concentration (ng/ml)	% Unspiked folate concentration	
	Radioimmunoassay	Competitive binding assay
1	98.7 ± 16.5	126.1 ± 15.7
10	98.5 ± 26.1	219.5 ± 38.5
100	94.3 ± 18.1	562.5 ± 88.3

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(0.03%), folic acid (0.3%) and N^{10}-methylene tetrahydrofolic acid (0.01%). The addition of ZD1694 at concentrations likely to be present in human plasma following its administration (3 mg/m^2 once every 3 weeks) caused overestimation of plasma folate levels when the samples were assayed using the competitive binding assay. There was no apparent interference in these samples when a radioimmunoassay was used. As several novel antifolates are presently being studied clinically, this potential drug interaction should be investigated further and taken into account in the evaluation of folate levels in patients being treated with these and other antifolates.

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