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Letters

The Microtitre Succinate Dehydrogenase Inhibition Test for Chemosensitivity of Human Tumour Cells

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THE succinate dehydrogenase inhibition (SDI) test is a simple, rapid and inexpensive test to determine chemosensitivity of tumour cells to anticancer drugs. It is based on the correlation of succinate dehydrogenase (EC 1.3.99.1) activity with cell viability [1, 2] and shares a common principle with the MTT dye assay [3]. We have developed and evaluated a microtitre test, and report our findings with this micro test compared with the SDI test.

The macro test was done as described [1, 2]. For the micro test, the SDI test was modified as follows: firstly, single cell suspensions were obtained by enzymatic disaggregation of solid tumours with 0.2% pronase, 0.25% collagenase and 0.1% DNase for 20 min at 37°C, which gave a higher cell yield and viability compared with mechanical disaggregation used in the macro test; secondly, the formazan crystals were dissolved with dimethyl sulphoxide, giving a higher absorption peak, with a microtitre plate spectrophotometer (Easy Reader EAR340, SLT Labo-instruments, Austria).

Human cancer tissues from 16 patients (Table 1) were used for both tests. We confirmed that absorbance of the formazan product was proportional to the number of tumour cells (10^3–10^5 cells per well in the micro test). To assess chemosensitivity, the micro SDI test required 2–5 × 10^4 tumour cells per well, one-tenth of that needed for the macro test. Good correlations were found (Table 1) between the micro and the macro SDI tests in the succinate dehydrogenase activity of the tumour cells (r = 0.767–0.999) exposed to 6 anticancer drugs: carboquone (0.5 μg/ml), doxorubicin (4 μg/ml), mitomycin (10 μg/ml), aclarubicin (4 μg/ml), cisplatin (20 μg/ml) and 5-fluorouracil (100 μg/ml). Chemosensitivities assessed by the micro and macro SDI tests were in general agreement when the drug sensitivity in both tests was arbitrarily defined as a 50% or greater reduction of succinate dehydrogenase activity in the presence of drug compared with its absence.

Therefore, the micro SDI method facilitates testing of a large number of drugs with minimal specimens, and may replace the SDI test for chemosensitivity testing of clinical tumour cells.

Table 1. Correlation between micro and macro SDI tests for chemosensitivity of 16 human cancer tissues exposed to 6 anticancer drugs

No. of cases	Tumour type	Correlation coefficient
5	Thyroid	0.767–0.942
4	Lung	0.796–0.971
4	Colon	0.889–0.999
3	Gastric	0.811–0.960

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Phase II Trial of Fotemustine in Advanced Colorectal Cancer

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NITROSOUREAS MAY play a determinant role in the activity of combinations such as lomustine vincristine and fluorouracil (MOF), and streptozocin (MOF-strep); with an estimated response rate up to 30% when administered as adjuvant treatment and a benefit on survival [1–3]. Fotemustine, a new nitrosourea derivative, has definite activity in malignant melanoma and primitive or metastatic brain tumours [4, 5]. When linked to aminophosphonic acid used as a carrier, an optimal partition coefficient is achieved, facilitating distribution among tissues and passage across cell membranes and the blood–brain barrier. We report our study of fotemustine in advanced colorectal cancer.

Patients with proven, measurable and progressive non-pretreated colorectal cancer were eligible for the study. From July to December 1988, fotemustine was administered weekly for 3 weeks (100 mg/m^2 induction) as an intravenous infusion over

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1 h. Evaluation of the response on day 21 and 49 included chest X-ray, computerised tomography for abdominal masses, and ultrasound for liver metastases [6] or peripheral lymph nodes. Toxicity was given WHO grading. On day 49, patients with non-progressive disease received fotemustine (100 mg/m^2 maintenance) given every three weeks until disease progressed or cumulative toxicity developed.

Of the 16 patients who entered the study, 2 were not evaluable; 1 because of hepatic dysfunction at entry, the other because of non-compliance during the induction cycle. 10 patients were male and 4 female; median age was 54 (range 45–76) years and median Karnofsky score was 80% (range 50–100). The metastatic sites were: liver (5), liver and other sites (4), lung (3) and lymph nodes (2). The overall response rate was 1 of 14. 1 patient with metastatic subclavicular lymph nodes had a partial response for 1 year; another had a minor response in hepatic lesions for 9 months. 2 patients were stabilised for 4 months, and 10 patients had progressive disease (1 had a partial response in a cutaneous site).

Toxicity was mainly haematological. The median value of the nadir for leucocytes and thrombocytes was 1.5 (0.8 to greater than 4) $\times$ 10^9/l and 30 (12 to greater than 100) $\times$ 10^9/l, respectively. Grade 4 thrombocytopenia occurred in 6 of 13 patients but none required platelet transfusion. The median nadir for thrombocytopenia was on day 35 and for leucopenia on day 49. Recovery was achieved within 8 days. The high toxicity against platelets was surprising. In comparison with previously reported results, the only difference in our study, apart from a small sample which may cause statistical insignificance, was tumour type. Reports on activity and toxicity of fotemustine in different tumour types show substantial variability in haematotoxicity: less than 10% grade 4 leucopenia and thrombocytopenia in malignant melanoma and primitive brain tumours; and 20% leucopenia and 30% thrombocytopenia grade 4 in non-small cell lung carcinoma, suggesting different susceptibility according to histological type [4, 5, 7, 8].

No delay was observed during induction and no dose reduction made during induction or maintenance therapy. Of the 5 patients given maintenance treatment, 3 had a 1–3 week delay because of prolonged neutropenia, and 1 had to wait 3 months because of prolonged hyperbilirubinaemia. Hepatic toxicity with a transient increase in transaminases (WHO grade 2) was observed in 3 patients, and in 1 patient it was accompanied by an increase in alkaline phosphatase (grade 2) for 1 month and hyperbilirubinaemia (grade 4) for 3 months. This jaundice episode was documented by a transcutaneous liver biopsy showing chemical hepatitis. As the patient had a minor response of liver metastases, he received one administration of fotemustine as first maintenance treatment, with no major adverse effect; however, treatment was discontinued because of further progressive disease. Nausea and vomiting were moderate, 30% of drug administrations giving a toxicity grade 2 or 3, with prophylactic doses of antiemetics.

With 1 response out of 14 patients, our results are similar to the previously reported activity of nitrosoureas in advanced colorectal cancers [9]. With the intra-arterial hepatic route, a response rate of 20% has been observed with fotemustine alone for liver metastases of colorectal origin [10]. Haematological toxicity may be severe, but digestive tolerance, compared with other nitrosoureas, is higher and mutagenicity in experimental models is lower [11]. These data and the possibility to overcome the mechanisms of resistance to nitrosoureas justify further investigation of fotemustine in colorectal cancer.

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Adult T-cell Leukaemia/Lymphoma and Horizontally Transmitted Human T-lymphotropic Virus Type I

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HUMAN T-LYMPHOTROPIC virus type I (HTLV-I) carriers vertically infected from their mothers are at risk of adult T-cell leukaemia/lymphoma (ATL) [1]. However, it is not known whether virus carriers horizontally infected through sexual contacts or through transfusion are similarly at risk [2].

If female HTLV-I carriers infected by sexual transmission from male partners are at risk of ATL, discrepancies by gender will exist in the age at onset, age distribution and other related epidemiological variables. However, the mean age at onset of ATL among females is greater than that of males, but statistically insignificant [3]. Two density function variables, according to the Weibull distribution, among females were almost identical to those of males, which suggests no difference, by gender, in leukemogenesis [4]. Moreover, the latency period from infection

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