Eur J Cancer, Vol. 26, No. 11/12, p. 1260, 1990.
Printed in Great Britain
0277-5379/90 $3.00 + 0.00

Letters

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

Shunji Kohnoe, Yasunori Emi, Yoshihisa Sakaguchi, Yoshihiko Maehara, Tetsuya Kusumoto and Keizo Sugimachi

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

Correspondence to S. Kohnoe.
S. Kohnoe, Y. Emi and Y. Sakaguchi are at the Cancer Center of Kyushu University Hospital, and Y. Maehara and K. Sugimachi are at the Department of Surgery II, Faculty of Medicine, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812; and T. Kusumoto is at the National Kyushu Cancer Center, Fukuoka 815, Japan.

1. Maehara Y, Kusumoto H, Kusumoto T, Anai H, Sugimachi K. Tumor tissue is more sensitive to mitomycin C, carboquone, and aclacinomycin A than is adjacent normal tissue *in vitro*. *J Surg Oncol* 1989, **40**, 4–7.
2. Maehara Y, Kohnoe S, Sugimachi K. Chemosensitivity test for carcinoma of digestive organs. *Semin Surg Oncol* 1990, **6**, 42–47.
3. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Meth* 1983, **65**, 55–63.

Eur J Cancer, Vol. 26, No. 11/12, pp. 1260–1261, 1990.
Printed in Great Britain
0277-5379/90 $3.00 + 0.00
Pergamon Press plc

Phase II Trial of Fotemustine in Advanced Colorectal Cancer

H. Bleiberg, D. Becquart, J. Michel, F. Cavalli and B. Gerard

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

Correspondence to H. Bleiberg.
H. Bleiberg is at the Department of Gastroenterology, Institut J. Bordet, Bvd de Waterloo 125, D. Becquart is at U.Z.A., Antwerp, J. Michel is at the Centre Hospitalier de Tivoli, La Louvière, and B. Gerard is at the Institut de Recherches Internationales Servier, Brussels, Belgium; and F. Cavalli is at the Ospedale San Giovanni, Bellinzona, Switzerland.