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# A comparison of a CB17 *scid* mouse model and the tetrazolium-dye assay using human haematological tumour cell lines

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**Abstract** Haematological tumours in the CB17*scid* mouse produce a disseminated blood-borne disease analogous to that seen in humans. The CB17*scid* mouse model has been applied to study the efficacy of chemotherapeutic agents on tumours. Using three human tumour-cell lines of haemopoietic origin (CCRF-CEM, Raji, HS-Sultan), we established disseminated tumours in *scid* mice and studied the in vivo response of these tumours to four chemotherapeutic agents (daunorubicin, idarubicin, ifosfamide, etoposide). The in vitro drug-resistance profiles of the same cell lines to these drugs were also determined by the tetrazolium-dye (MTT) assay. Differences were found in the patterns of resistance and sensitivity of the cell lines in the in vivo and in vitro systems tested. Since the *scid* mouse model determines the in vivo response of both host and tumour to cytotoxic agents, it may be more valid than the other models in determining drug resistance of haematological malignancies.

**Key words** Drug resistance · Leukaemia · MTT · Myeloma · *Scid* mice

**Abbreviations** *ALL* Acute lymphoblastic leukaemia, *HSA* human serum albumin, *MTT* 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, *Scid* severe combined immunodeficiency

## Introduction

For decades, methods for assessing in vitro sensitivity/resistance of tumour cells to chemotherapeutic agents have been studied with the view of developing systems similar to bacteriological culture and sensitivity tests in clinical practice. Various types of assays, including clonogenic and dye-exclusion assays and radioactive precursor incorporation, have been described. Each assay has its pros and cons but most of these methods are time-consuming, labour-intensive and subject to observer error. In 1983 a semiautomated short-term culture assay was described by Mosmann [1] on the basis of the ability of viable cells to reduce a tetrazolium salt, 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). This MTT assay has been successfully applied by the National Cancer Institute in the United States to determine the sensitivity/resistance of tumour cell lines to new chemotherapeutic agents [2]. The in vitro cytotoxicity assays have also been used to select the most effective agents for clinical use by several groups [3, 4].

The congeneic inbred strain CB17*scid* mouse (*scid*) was derived from progeny of low-IgM-producing individuals of CB17, which were subsequently found to be T-cell deficient [5]. This mouse strain has been used successfully to support transferred xenogeneic cells from bone marrow of leukaemic patients and normal individuals [6–8]. Human monoclonal antibodies have been engineered from a splenic library of a humanised *scid* mouse [9]. Several laboratories have reported that *scid* mice are capable of producing antigen-specific human antibodies from peripheral blood mononuclear cells transferred i.p. to the mouse [10–12].

An in vivo model such as *scid* mice for determination of cellular resistance to anticancer agents is a complex biological system and requires validation. The *scid* mouse model has been used for in vivo screening of drug resistance of human lung-cancer cell lines [13]. We report as a parameter of in vivo chemosensitivity the clinical outcome of *scid* mice inoculated with tumour cell lines and subsequently injected with cytotoxic drugs, and we also compare the in vivo

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results with the in vitro data on these same cell lines derived from the MTT assay.

## Materials and methods

### Cell lines

CCRF-CEM is a T-lymphoblastoid cell line derived from a patient with acute T-lymphoblastic leukaemia (T-ALL). Raji is an Epstein-Barr nuclear antigen-positive ($EBNA^+$) lymphoblast-like cell line derived from a patient with Burkitt's lymphoma, and HS-Sultan is an $EBNA^+$ cell line secreting IgG-kappa immunoglobulin that was derived from a patient with multiple myeloma. All cell lines were obtained from the American Tissue Culture Collection repository. These cell lines were reseeded periodically from frozen stocks of the originally received line.

### *Scid* mice

A breeding nucleus was obtained from the Medical Research Council (Millhill, London) and maintained in a positive-pressure, HEPA-filtered isolator (Moredon Ltd, Edinburgh). Subsequent stock were maintained by sibling mating. A second isolator was used to maintain the experimental mice. Both groups of mice were given autoclaved water and irradiated food ad libitum. Prior to the start of the study, serum IgM levels were checked in all mice using a modified catchment enzyme-linked immunosorbent assay (ELISA) developed in this department [14]. Only mice with IgM and IgG values of $< 1$ μg/ml were entered in the study.

### Cytotoxic reagents

The most clinically relevant drugs for the lymphoid lineage were chosen. All cytotoxic drugs given to *scid* mice were obtained from the Pharmacy, Royal Victoria Infirmary, and were used within 12 h of their dispensation. For in vitro use, daunorubicin and etoposide were purchased from Sigma, idarubicin was a gift from Farmitalia Carlo Erba (Milton Keynes, UK) and 4-hydroperoxy-ifosfamide (an active metabolite of ifosfamide) was kindly provided by Dr. Dianne Walker (Pharmacogenetics, Newcastle University).

### MTT assay

Cells were suspended at a concentration of $2 \times 10^6$/ml in RPMI 1640 containing 15% (v/v) fetal calf serum supplemented with 2 m*M* L-glutamine, 25 U penicillin/ml and 25 μg streptomycin/ml, and 80 μl (in duplicate) was added to each well of a 96-well U-bottom microculture plate. The wells of this plate contained various concentrations (see below) of cytotoxic drugs. After incubation of the plate for 4 days in humidified air enriched with 5% $CO_2$ at 37 °C, 10 μl MTT solution (5 mg/ml) was added. The MTT was reduced to a coloured formazan product by living cells, and after 4 h the formazan crystals were dissolved in 100 μl dimethylsulfoxide. The optical density of the formazan solution was measured with a microplate reader (Titertek Multiskan 330). The optical density is linearly related to the number of viable cells. The $IC_{50}$ was the drug concentration at which 50% of the control cells were inhibited. The concentrations of drugs tested were 0.002–2 μg/ml for daunorubicin and idarubicin, 0.1–100 μg/ml for 4-hydroperxy-ifosfamide and 0.01–100 μg/ml for etoposide.

**Table 1** Survival in days of *scid* mice inoculated i.v. with $5 \times 10^5$ cells of each tumour cell line (*n* Total number of mice, *SD* standard deviation)

| | CCRF-CEM | Raji | HS-Sultan |
|---|---|---|---|
| Mean | 37 | 21 | 32 |
| Experiments | 10 | 10 | 9 |
| *n* | 20 | 20 | 18 |
| SD | 4.5 | 2.0 | 4.6 |

### *Scid* mouse assay

Mice were injected i.v. with $5 \times 10^5$ cells from each cell line. This number produced a lethal disease in 100% of the animals within the times indicated in Table 1. Further details of these cell lines and of the pattern of disease in *scid* mice have been reported elsewhere [14]. Drugs were diluted in 5% human serum albumin/phosphate-buffered saline (HSA-PBS), and 100 μl was injected intravenously at 48 h after the inoculation of cells. The doses of cytotoxic drugs were ascertained from the maximum tolerated doses in mice as determined in our laboratory (1.5 mg/kg for daunorubicin, 1.5 mg/kg for etoposide, 120 mg/kg for ifosfamide with 120 mg/kg mesna, 0.5 mg/kg for idarubicin). Mice were culled according to Home Office guidelines when frank disease was evident; this time point was taken as the survival duration.

## Results

The response of *scid* mice to inoculation with each cell line was found to be reproducible in ten separate experiments (Table 1). The mean survival times were 37 (SD 4.5) days for CCRF-CEM, 21 (2.0) days for RAJI and 32 (4.5) days for HS-Sultan. These figures are derived from the cumulative experiments of all the controls for the various drugs used as shown in Table 2, with survival time following a Gaussian distribution.

Table 2 shows the in vivo response of *scid* mice inoculated with tumour cells and subsequently treated with cytotoxic agents. Daunorubicin, idarubicin and etoposide produced no improvement in survival as compared with control mice inoculated with CCRF-CEM and Raji cell lines. The response to ifosfamide, however, was different, as both cell lines showed in vivo sensitivity. For HS-Sultan a statistically significant difference was found in mean survival between test and control mice for daunorubicin, idarubicin and etoposide.

The in vitro drug sensitivity of these tumour cell lines for the same drugs is shown in Table 3. CCRF-CEM was least resistant among the cell lines to all of the drugs tested, and the $IC_{50}$ values were within the range of concentrations found in blasts from patients with ALL [15]. Raji cells were highly resistant to all drugs tested, whereas the $IC_{50}$ values found for HS-Sultan were intermediate between those noted for CCRF-CEM and those recorded for Raji.

**Table 2** Survival in days of *scid* mice treated with chemotherapy following their inoculation with tumour cell lines. Mice were inoculated i.v. with $5 \times 10^5$ cells and then given a single i.v. bolus of the chemotherapeutic agent as outlined in Materials and methods. (95% *CI* 95% Confidence interval for the population median – range 96.1– 99.2%, *crosses* total range – 95% CI cannot be calculated, *n* number of mice in each group, *NS* not significant, *P* level of significance as determined by Mann-Whitney *U*-test)

| | | Daunorubicin | | Idarubicin | | Etoposide | | Ifosfamide/mesna | |
|---|---|---|---|---|---|---|---|---|---|
| | | Control | Test | Control | Test | Control | Test | Control | Test |
| CCRF-CEM | Median | 37 | 37 | 39 | 41 | 36.5 | 39.5 | 32 | 38.5 |
| | 95% CI | 32–44 | 32–44 | 32–41 | 34–48 | 33–48 | 34–46 | 31–33† | 37–43† |
| | *n* | 6 | 10 | 6 | 8 | 6 | 10 | 2 | 4 |
| | *P* | NS | | NS | | NS | | 0.07 | |
| Raji | Median | 22 | 22 | 21.5 | 21.5 | 20 | 22 | 23 | 26 |
| | 95% CI | 20–27 | 20–43 | 19–27 | 18–32 | 20–27 | 19–24 | 23–23† | 24–28† |
| | *n* | 6 | 10 | 6 | 10 | 6 | 9 | 2 | 4 |
| | *P* | NS | | NS | | NS | | 0.07 | |
| HS-Sultan | Median | 31 | 35 | 30.5 | 33 | 35 | 42 | 32 | 48.5 |
| | 95% CI | 28–35† | 31–46 | 24–33† | 33–45 | 33–42† | 38–62 | 30–34† | 40–51† |
| | *n* | 4 | 10 | 4 | 9 | 4 | 7 | 2 | 4 |
| | *P* | < 0.1 | | 0.05 | | 0.02 | | 0.07 | |

**Table 3** $IC_{50}$ values, expressed in μg/ml, of cell lines to cytotoxic agents tested by the MTT assay. The $IC_{50}$ values were determined by calculation of the mean value from triplicate experiments. The coefficient of variation of $IC_{50}$ values for each drug was less than 5%

| | Dauno-rubicin | Idarubicin | Etoposide | 4-Hydroperoxy-ifosfamide |
|---|---|---|---|---|
| CCRF-CEM | 0.067 | 0.046 | 0.174 | 7.23 |
| Raji | 2 | 0.607 | 52.75 | 15.21 |
| HS-Sultan | 0.29 | 0.307 | 0.912 | 10.84 |

## Discussion

The *scid* mouse model may simulate a true biological interaction of leukaemic process, host response and chemotherapeutic intervention. The three cell lines used produced a pattern of disease in *scid* mouse analogous to that seen in humans with disseminated tumour relevant to the cell line. Both CCRF-CEM and Raji cell lines invaded the liver, spleen and bone marrow and also affected the nervous system, causing hind-limb paralysis. HS-Sultan produced a solid i.p. tumour and ascites in mice passaged i.v. but only rarely affected the nervous system [14]. As such the *scid* mouse model is better than the nude mouse model for the study of leukaemia, lymphoma and myeloma.

Although the overall numbers of mice in each experimental group were small, the survival duration of control mice for each cell line was reproducible (Table 1). The MTT assay gave $IC_{50}$ values for CCRF-CEM within the range of values seen in ALL cells from patients who have responded favourably to chemotherapy [16]; therefore, the in vitro results indicated that CCRF-CEM are chemosensitive. This result contrasts with the in vivo findings in mice, where CCRF-CEM cells were resistant to the chemotherapeutic agents tested, resulting in no improvement in survival of the mice. It may well be that these drugs were given to mice in doses lower than those that would be effective in increasing survival. The doses are, however, the maximum tolerated in *scid* mice as determined from our preliminary experiments. On the basis of body surface area, the doses also approximated those given for the relevant disease in humans. The discrepancy between the *scid* mouse model and the MTT assay in determination of drug sensitivity highlights the drawback of the in vitro cytotoxicity assays, which do not take pharmacokinetic variables into account. There is no evidence that humans and *scid* mice handle the drugs in the same manner, and detailed pharmacokinetics studies are required to determine the optimal administration of drugs in the *scid* mouse model.

Despite discrepancies in the results noted for CCRF-CEM, the Raji cell line showed concordant results between in vitro and in vivo systems. This may reflect that in vitro cytotoxicity tests are more sensitive in predicting chemoresistance as opposed to chemosensitivity. The MTT assay is also affected by cellular proliferation. CCRF-CEM cells have a lower in vitro proliferative capacity than HS-Sultan and Raji cell lines, and this may explain why CCRF-CEM cells are sensitive in vitro but resistant in vivo to chemotherapy.

The HS-Sultan cells were less resistant than CCRF-CEM cells to chemotherapy in *scid* mice, although the former were more resistant in the MTT assay. This suggests that in vitro $IC_{50}$ values vary widely depending on the tumour type and that they should not be referred to interchangeably among different cell lines for the prediction of chemosensitivity. It is therefore difficult to compare the chemosensitivity of different cell lines by comparing the $IC_{50}$ values determined by the MTT assay. The usefulness of this assay may be limited to comparing the resistance profiles of a parent

cell line versus a daughter cell line. This is one reason why the cytotoxicity assays have not been successful in selecting the most effective agents for treatment of leukaemia. In ALL, the MTT assay has failed to predict the most effective agents for tailor-made chemotherapy [4], although it is a useful prognostic test [15, 17].

Our findings may suggest that the *scid* mouse model is better than the in vitro cytotoxicity assays in prediction of drug resistance of tumour cells. Since this is a true biological system, it is subject to variations, and this may be one reason why the response of the cell lines to cytotoxic agents in the mice was not in total concord with the pattern of response of the analogous tumours to chemotherapy seen in humans. By definition, *scid* mice are immunodeficient and are therefore prone to infective and immunological insults that could adversely affect the animal's ability to handle drugs. Since these mice are kept under positive air pressure and in sterile conditions, they rarely succumb to opportunistic infections, which would tend to rule out this possibility. The role of activated lymphocytes in promoting an antitumour effect has been well documented [18, 19]. The immune system is impaired in *scid* mice and, thus, tumours could be more aggressive and less amenable to cell kill by cytotoxic agents in these animals than in humans. Nonetheless, several groups have reported that transfer of human cell lines derived from haematological malignancies is capable of producing disseminated disease in the *scid* mouse model similar to the disease pattern shown in humans [20–22]. The model has also been used to measure the efficacy of treatment with single-agent [23] and combination [24] chemotherapy and also with immunotoxins [25–27].

The *scid* mouse model also takes account of the biological behaviour of the tumour cells tested. In our experiment model, HS-Sultan, unlike the other two cell lines, did not seem to invade the so-called *sanctuary* sites such as the nervous tissue. This could explain why the other two cell lines were more resistant in vivo to chemotherapy, allowing tumours to re-establish from these sites. Cranial irradiation is part of the routine therapy given to children with ALL, as it has been shown that many of them otherwise relapse with tumours in the central nervous system. CCRF-CEM was isolated from a 4-year-old girl with ALL and has been shown by us to produce tumour foci in the *scid* mouse forebrain [14]. The biophysical properties of the drugs tested are also reflected in the *scid* mouse model but not in the in vitro cytotoxicity assays. For example, we have shown that melphalan is capable of lengthening the survival of *scid* mice inoculated with CCRF-CEM [14]. Melphalan has been detected in the cerebrospinal fluid (CSF) at approximately 10% of the plasma concentration following moderately high to high i.v. dosage [17] and, thus, could contribute to the clearance of leukaemia from sanctuary sites. The sensitivity of tumour cell lines of ifosfamide in the study may also be explained in a similar manner, as the mean CSF-to-plasma concentration of alkylating activity after high-dose ifosfamide therapy has been reported to be as high as 0.53 [28].

In conclusion, the usefulness of in vitro cytotoxicity assays has limitations. The *scid* mouse model is closer to the clinical situation and takes into account some of the pharmacokinetic variables, biophysical properties of the drugs and biological characteristics of tumour cells. In particular, the *scid* mouse system would be more suited for testing of agents that require activation in the host, for example, drugs such as ifosfamide reported herein, which is inert as such and is converted to its active metabolites by the liver. The model, however, takes several weeks to produce results and therefore unsuitable for testing of pathological material from patients for the prediction of drug resistance with a view towards tailor-made chemotherapy or risk-group stratification for optimal therapy.

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