Reducing the Cost of Screening Novel Agents Using the Hollow Fibre Assay

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Abstract. *Although the in vivo hollow fibre assay (HFA) as utilised by the National Cancer Institute is a highly effective screening tool, it has not been adopted en masse in the cancer pharmacology field. However, in laboratories which have adopted it, the effectiveness of HFA has also been confirmed. If immunocompetent mice could be used with the HFA, thereby reducing the cost of the assay, accessibility would increase and reductions in the cost of selecting appropriate agents for early clinical trials would result. It was demonstrated here that there was no difference in terms of cell growth and response to chemotherapy for cancer cells in hollow fibres in immunocompetent compared with immunodeficient mice. The HFA can thus be performed in these less expensive and more easily available mice with the implication of considerable savings to the preclinical cancer pharmacology community.*

The *in vivo* hollow fiber assay (HFA) was implemented by the US National Cancer Institute (NCI) in the mid-1990s as a preliminary rapid screen for assessing novel potential anti-cancer agents prior to xenograft model evaluation (1). In the assay, human tumour cell lines are loaded into biocompatible polyvinylidene fluoride (PVDF) hollow fibres (HF) with a 500 kDa molecular weight exclusion, which allows free passage of macromolecules and drugs, while restricting the passage of cells. These HF are transplanted subcutaneously (*s.c.*) and intraperitoneally (*i.p.*) into immunocompromised nude mice and, following treatment, the cells are harvested and then analysed for viability. The number of viable cells in the HF from treated animals are compared with the untreated controls. Extensive analysis has demonstrated that the HFA is a good indicator of potential *in vivo* response, thus validating the effectiveness of the assay (2, 3) .

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Key Words: Drug evaluation, preclinical, hollow fibres.

Although the HFA is a highly effective screening tool, it has not been adopted en masse in the cancer pharmacology field. There are a number of reasons for this, the major one being that it is assumed that, as with xenograft assays, immunocompromised mice are required, and this is expensive in terms of specialist barrier facilites required to keep such mice, as well as their relative cost compared to immuno-competent mice. The NCI uses immunocompromised mice for the HFA and, although the numbers of mice used are considerably fewer than those necessary for conventional xenograft studies, it is still a substantial number. The use of syngeneic tumours also requires many more mice than would be used with the HFA and does not present a human tumour target for potential human tumour treatment. If immunocompetent mice could be used with the HFA, the reduction in the cost of the assay would increase its accessibility and lead to an increase in the numbers of agents going through early preclinical study, thus hastening the selection of agents for early clinical trials.

Previous studies have reported the use of the HFA with immunocompetent mice or rats for analysis of anticancer agents (4-8) and xenotransplantation of pancreatic islets (9). None of these studies compared whether the loaded tumour cells had the same characteristics in terms of growth and response to therapy as they would in immunodeficient animals.

Our aim was to establish whether human cells seeded in HF grow and respond to therapy in immunocompetent mice in the same way as when assayed in immunodeficient mice.

Materials and Methods

Compounds. Doxorubicin and paclitaxel were both purchased from Sigma (Poole, UK). For the therapeutic response studies, doxorubicin was dissolved in saline and paclitaxel was dissolved in 10% DMSO: 10% Cremophor EL: 80% saline.

Cell lines. Three human tumour cell lines were selected for analysis: DLD-1 colon adenocarcinoma, H460 non-small cell lung carcinoma, MCF-7 mammary adenocarcinoma (all from LGC Promochem, Middlesex, UK). The cells were cultured in RPMI 1640 cell culture

medium supplemented with 1 mM sodium pyruvate, 2 mM L-glutamine and 10% foetal bovine serum (all from Sigma) and were maintained as monolayer cultures at 37˚C in a humidified 5% CO_2 environment.

Animals. Female NMRI immunocompetent and CD1-Foxnl^{nu} immunodeficient nude mice, aged 6-8 weeks, were used (B and K, Hull, UK and Charles River Laboratories, Margate, UK, respectively). The mice were kept in cages housed in isolation cabinets in an air-conditioned room with regular alternating cycles of light and darkness. They received Teklad 2018 diet (Harlan, Blackthorn, UK) and water *ad libitum*. All animal procedures were carried out under a project licence issued by the UK Home Office and UKCCCR guidelines were followed throughout (10).

Hollow fibre assay. The cells were loaded into sterilised colour-coded PVDF Spectra/Por hollow fibres (Spectrum Medical Inc, Houston, TX, USA) as described previously (11). Briefly, the cells were harvested and resuspended in cell culture medium at the required cell density. This preparation was then loaded into the HF and the ends were clamped and heat-sealed. The HF was then cut into 1.5-cm lengths, which were again heat-sealed at both ends, followed by transfer to 6-well plates containing medium before implantation.

Under brief inhalation anaesthesia (2% isoflurane), one loaded HF for each of the cell lines was transplanted *i.p.* or *s.c.* into the dorsal flank of each mouse and the mice were allowed to recover. For growth assessment, three mice were sacrificed on days 3, 5 and 7 post-implantation, the fibres were removed and cell growth was analysed using a modified MTT assay (1). For assessment of therapeutic response, groups of five mice were treated with either 2.5 mg/kg^{-1} doxorubicin intravenously (*i.v.*), or 10 mg/kg^{-1} paclitaxel *i.p.* on days 0, 1, 2 and 3 post-implantation. The mice were sacrificed on day 6 post-implantation, the fibres were removed and cell survival was analysed using a modified MTT assay, comparing absorbances seen for the treated groups with an untreated control group. The statistical analysis was carried out using a multiple linear regression model.

Results

Cell growth comparisons. All three cell lines grew in the implanted fibres in both strains of mice at both sites (Figure 1). When subcutaneously implanted HF were examined *in situ*, there was no difference in the appearance of the tissue surrounding the HF. The best growth was seen for DLD-1 and H460, while the absorbances seen for MCF-7 cells were slightly lower. Statistical analysis revealed that there was no significant difference by implantation site between the immunocompetent and immunodeficient mice ($p=0.46$).

Evaluation of therapeutic response. The responses to therapy in terms of cell survival for the three cell lines are illustrated in Figure 2. The absorbances obtained for cell growth in the untreated control groups were similar to those obtained in the growth experiment, with DLD-1 and H460 cells demonstrating the best growth. In terms of the response to therapy seen for the two standard agents, again there was no significant difference between the mouse strains ($p=0.51$). The best response to therapy was seen with paclitaxel for all three cell lines transplanted *i.p.* Significant therapeutic effects in terms of reduction in percentage cell survival were seen for both compounds at both implantation sites for the H460 and MCF-7 cell lines. In the DLD-1 cell line, no significant therapeutic effects were seen for doxorubicin at either implantation site or for paclitaxel at the *s.c.* site.

Discussion

The findings of this study suggest that there is no disadvantage to using immunocompetent mice for the short-term HFA as opposed to the immunodeficient mice currently used. These findings are not surprising on taking into consideration the results of previous studies involving the placement of human material in immunocompetent mice using the subrenal capsule assay (12). Here, a 6-day protocol was used in order to avoid the period of greatest immunological regression, which occurs 6-12 days post-implantation (13, 14).

Further studies in this laboratory using A549 non-small cell lung carcinoma, HT-29 colon adenocarcinoma and HL-60 myeloblastic leukaemia cell lines also showed no difference in growth rates between immunocompetent and immunodeficient mice (data not shown), thus further reinforcing the findings that the lack of difference between strains in not cell-specific.

The reduced growth seen for the MCF-7 cell line compared with the other two cell lines is possibly due to the fact that supplementary oestrogen (normally administered when growing MCF-7 *in vivo*) was not included in the experimental protocol due to the potential of affecting the growth properties of the mixture of cell types used.

When the response rates to treatment with doxorubicin and paclitaxel were analysed, it was seen that there was no difference in the rates between the two strains of mice. The activity of both agents was less in the DLD-1 cell line compared with the other cell lines tested, as would be expected based on NCI screening data and the study by Toffoli *et al.* (15), where DLD-1 cells were not as sensitive to the two agents compared with the other two cell lines. The fact that the best chemosensitivity was observed with *i.p.*-administered paclitaxel in the *i.p.*-implanted cells was expected as the cells were directly exposed to the agent administered by this route. In contrast, doxorubicin was administered *i.v.* and, hence, the pharmacokinetic profile would have meant that there would be no advantage in drug delivery to *i.p.* or *s.c.* sites, as was seen by the similarity in effect.

In conclusion, it was demonstrated that there was no difference in terms of cell growth and response to chemotherapy for cells in HF in immunocompetent

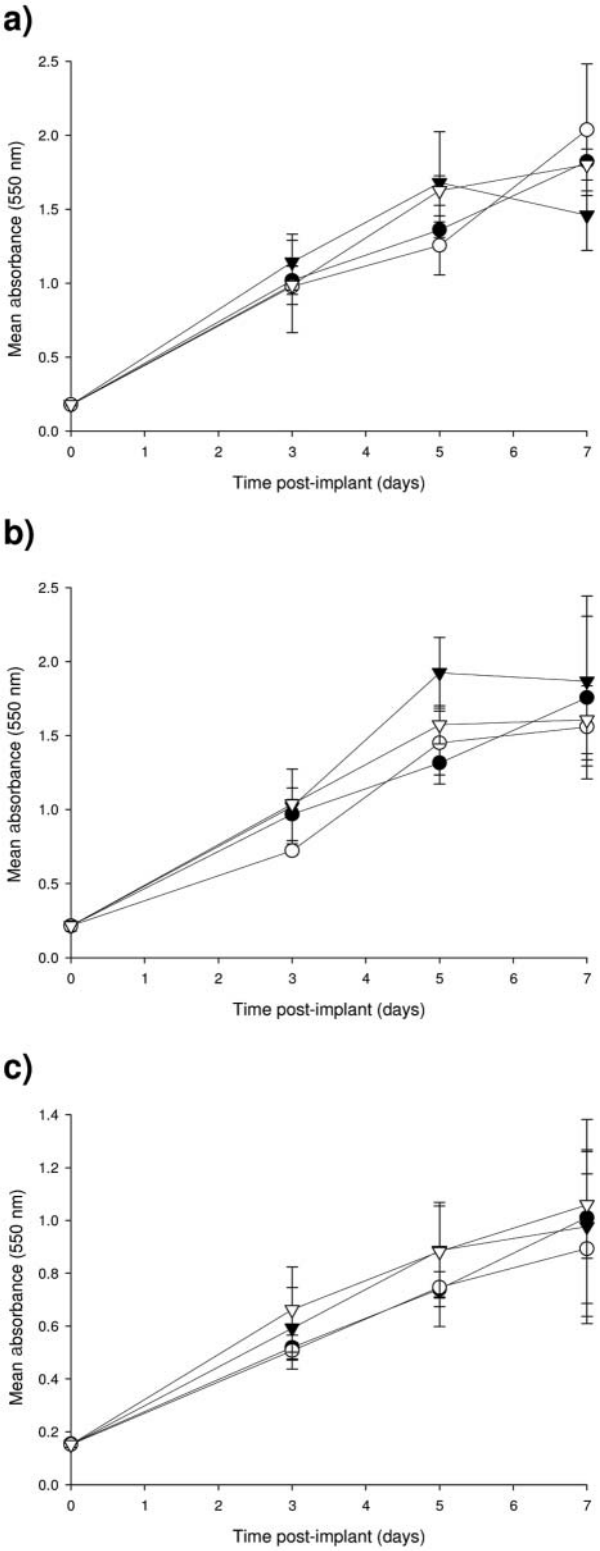

Figure 1. *Growth curves for tumour cells loaded in HF transplanted either s.c. or i.p. in immunocompetent NMRI or immunodeficient CD1-Foxnl^{nu} nude mice. a) DLD-1, b) H460, c) MCF-7. Points represent mean ± S.D. (n=3)* ● *NMRI s.c.;* ○ *Nude s.c.;* ▼ *NMRI i.p.;* ▽ *Nude i.p.*

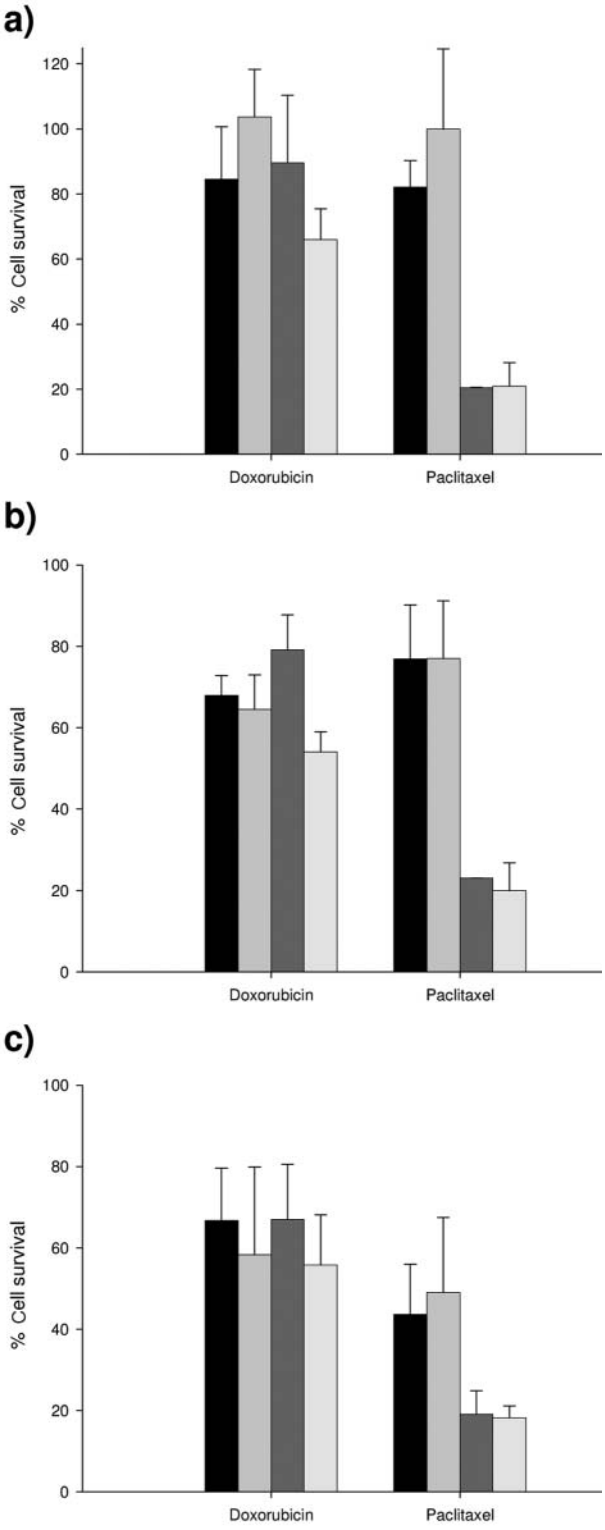

Figure 2. *Assessment of the effects of doxorubicin and paclitaxel on tumour cells loaded in HF transplanted s.c. or i.p. in either immunocompetent NMRI or immunodeficient CD1-Foxnl^{nu} nude mice. a) DLD-1, b) H460, c) MCF-7. Points represent mean ± S.D. (n=5)* ■ *NMRI s.c.;* ■ *Nude s.c.;* ■ *NMRI i.p.;* ■ *Nude i.p.*

compared with immunodeficient mice. Thus, the HFA can be performed in these less expensive and more readily available mice with the implication of considerable savings to the preclinical cancer pharmacology community.

Acknowledgements

We are grateful to Cancer Research UK for supporting this work through the following grants: C7589/A5953 and C7589/A5954.

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Received January 30, 2006
Accepted March 8, 2006