Microencapsulated Tumor Assay: New Short-Term Assay for in Vivo Evaluation of the Effects of Anticancer Drugs on Human Tumor Cell Lines

Eliaser Gorelik, Artemio Ovejera, Robert Shoemaker, Allan Jarvis, Michael Alley, Ronald Duff, Joseph Mayo, Ronald Herberman, and Michael Boyd

Abstract

A new in vivo assay has been developed for evaluating the antitumor activity of chemotherapeutic drugs. The assay is based on a microencapsulation technology developed by Damon Biotech, Inc., Boston, MA, which makes it possible to encapsulate human tumor cells in small (about 1 mm in diameter) microcapsules with semipermeable membranes. Microcapsules containing human tumor cells were injected i.p. into nude or C57BL/6 mice and drugs were administered i.v. The microcapsules were recovered at various intervals following treatment and determinations of drug effects were made based on the differences in the number of tumor cells recovered from the treated and nontreated animals. Using this assay we found that (a) encapsulated tumor cells grew better in the in vivo system than in vitro under the conditions tested; (b) drugs crossed the capsular membrane and killed or inhibited the proliferation of tumor cells; and (c) the antitumor effect was consistent with the relative therapeutic efficacy of drugs or level of resistance of tumor cells detected by other in vitro or in vivo tests. The tumor microencapsulation assay offers several properties which make it attractive for use in new drug development: (a) the antitumor activity of drugs can be tested against human tumor cells under conditions which provide for three-dimensional growth and in vivo supply of nutrients; (b) the sensitivity of tumor cells can be assessed following exposure to drugs at concentrations which are achievable in vivo; (c) compounds requiring in vivo metabolic activation can be tested; (d) the effect of each drug injection can be quickly evaluated; (e) inhibition of tumor cell proliferation versus cytoreductive effects of drugs can be discriminated; (f) the test is applicable to virtually all histological types of human tumor cells; and (g) the tumor microencapsulation assay is a short-term, simple, and relatively inexpensive assay.

Introduction

The use of in vivo murine leukemia models for large scale antitumor drug screening has been successful in identifying compounds with clinical activity (1-3). For the most part, however, this clinical activity has been limited to leukemias, lymphomas, and some rare solid tumors with very high growth fractions. In an effort to identify new compounds with potential activity against the common adult solid tumors (e.g., lung, colon, breast, prostate, etc.), the NCI has recently begun development of a new, disease-oriented approach to drug screening (4, 5). The approach is based on the use of multiple panels of human tumor cell lines representing various tumor types in a closely interrelated in vitro/in vivo screening paradigm. Each compound will be tested in vitro by using each cell line in the screening panel. Compounds showing differential cytotoxicity, or selective cytotoxicity for particular cell types, will then be subjected to in vivo follow-up testing with the use of sensitive cell lines identified in the in vitro testing. Reliable, short-term in vivo assays which would permit the individual study of all or most of the cell lines in the in vitro screening panel will be essential for such a large-scale screening program.

The availability of immunodeficient hosts (artificially immune suppressed or genetically immunodeficient nude mice) for xenografting of human tumors has made possible experimental therapeutic studies of human tumors in an in vivo environment. While the ultimate value of such models to cancer therapies remains to be fully elucidated, the use of human tumor cells in these models should potentially offer distinct advantages compared to the use of murine tumors (6). A wide variety of histological types of human tumors have been successfully propagated in nude mice. Typically, such xenografts have been established and maintained as s.c. tumors. Application of these s.c. models in large-scale drug screening has been hampered by the fact that many human tumors grow relatively slowly, requiring extended maintenance of animals and substantial expense. To circumvent this problem in the use of human tumors for drug testing there has been considerable investigation aimed at the development of short-term in vitro or in vivo assays (7-9).

The best known and most widely applied short-term in vivo assay uses the subrenal capsule model (9). While some controversy exists regarding this method (10-13), particularly a variant methodology using immunocompetent mice (14), many investigators have found the subrenal capsule assay to be useful for experimental chemotherapy studies. The National Cancer Institute has made extensive use of the assay with selected human tumor xenografts used for new drug screening. While the subrenal capsule assay may be suitable for many of the cell lines planned for use in the NCI disease-oriented screening program, some, such as very slow growing tumors or tumor cell lines which do not readily form s.c. tumors in nude mice, will present problems for this assay.

As an approach to development of an alternative short-term assay we have explored the use of a novel microencapsulation technology. Preliminary results with this META have been presented recently (15). The assay is based on a microencapsulation technology developed by Damon Biotech, Inc., Boston, MA. The technology makes it possible to encapsulate human tumor cells in small microcapsules which can be inoculated directly into the peritoneal cavity of mice. The semipermeable membranes of the microcapsules protect the encapsulated human tumor cells from destruction by the immune mechanisms of the host, but permit the flow of nutrients required for in vivo growth of tumor cells. Anticancer drugs may be administered systemically, then microcapsules are recovered and drug effects...
MATERIALS AND METHODS

**Mice.** Female C56BL/6 and athymic nude mice were obtained from the Animal Production Area (NCI-Frederick Cancer Research Facility, Frederick, MD). Mice were kept in specific pathogen-free conditions and were utilized for experiments when 2 to 4 months old.

**Tumors.** The following cultured human tumor cell lines were used: MOLT-4, T-lymphoma; HT-29, colon carcinoma; MCF-7, breast adenocarcinoma; A549, non-small cell lung cancer; and FEM, and LOX, melanoma cells. Murine P388 leukemia and a multidrug resistant variant selected in vivo by treatment with Adriamycin (P388/ADR) (16) were also tested. Each cell line was propagated in vitro by utilizing standard sterile culture techniques after recovery from cryopreserved (16) were also tested. Each cell line was propagated in vitro by utilizing standard sterile culture techniques after recovery from cryopreserved cell stocks. All cell lines were documented to be of human origin by karyotype and isoenzyme analysis and were free of adventitious bacteria and pathogenic virus (NCI-FCRF Diagnostic Microbiology Laboratory and Animal Health Diagnostic Laboratory). Cells were cultivated in T-flasks (75 cm²) in a standard culture medium, consisting of RPMI 1640 (Quality Biologicals, Inc., Gaithersburg, MD), 10% heat-inactivated fetal bovine serum (Sterile Systems HyClone, Logan, UT) and 2 mM L-glutamine (NCI-FCRF Central Medium Laboratory) without antibiotics at an initial density of 0.5 × 10⁶ cells/10 ml per flask. Cultures were maintained at 37°C, 5% CO₂, 100% relative humidity, and growth medium was replenished as necessary. Cell monolayers approaching 80% confluency were harvested by using the DeLarco formulation of trypsin/EDTA (NCI-FCRF Central Medium Laboratory), and were subcultured for a maximum of 8–12 serial passages following thaw.

**Microencapsulation Experiments.** Cells were harvested with trypsin/EDTA (NCI-FCRF Central Medium Laboratory), and were 80% confluent spheroid, whereas others form multiple spheroids or elipsoid masses inside individual microcapsules. These sphe-
capsules (approximately 200-500 per mouse) were recovered. The total packed volume of recovered microcapsules (ranging from 0.15 to 0.4 ml) was recorded for each mouse. The total volume of recovered microcapsules was almost completely filled by growing MOLT-4 lymphoma cells, whereas in ADR- or CTX-treated microcapsules the number of tumor cells was reduced by 95 to 97% relative to the control. However, treatment with 5-FUra was less effective since merely 30% inhibition of growth of encapsulated MOLT-4 lymphoma cells was found (Table 3). The variability in the number of tumor cells recovered from individual animals within treatment groups was relatively low (4–7% coefficient of variation).

To confirm and extend this latter observation, a study was conducted to determine within-group cell count variability with the use of a different tumor type (Table 4). One group of mice bearing microencapsulated LOX melanoma cells was given i.v. injections of ADR (6 mg/kg/injection) 1 and 4 days after microcapsule implantation; another group served as a nontreated control. Three mice from each group were sacrificed on days 6 and 8, microcapsules were recovered, and cell counts were made. LOX melanoma cells grew rapidly and in 6 days the number of tumor cells per unit volume of microcapsules increased approximately 20-fold. By day 6, the intracapsular space was almost completely filled with spheroidal tumor masses and tumor cell viability was below 50%. A slight increase in tumor mass was observed on day 8 accompanied by further decrease in cell viability. Treatment with ADR substantially inhibited tumor cell proliferation. A 90% growth inhibition was obtained after two treatments with the drug. Cell viability in the treated group was approximately 70%. Since the viability of tumor cells declined in saturated microcapsules, the total number rather than the number of viable tumor cells appeared to be a more appropriate indicator of tumor mass present inside the microcapsules. Thus, treatment effects were calculated based on the difference in total number of cells in treated versus nontreated groups. A very small range of variability in total number of cells recovered from individual mice within a treatment group was observed in this experiment. The coefficient of variations ranged from 3 to 5%. In the previous experiment with MOLT-4, low within-group variation was similarly obtained. This prompted us to conclude that for this assay 1–2 mice per group would be sufficient to give valid measurements. Moreover, it was deemed informative to evaluate drug effects at more than one time point. Using this type of protocol, the pattern of tumor growth at later stages after termination of treatment could be determined.

In further experiments the growth of encapsulated MOLT-4 lymphoma cells was assessed when drug treatment was postponed until 6 to 10 days after microcapsule implantation. The numbers of tumor cells were determined after the first and
second treatment (Table 5). One day after the first treatment with CTX or ADR there was some reduction, (17 to 35%, respectively) in the number of MOLT-4 cells. However, 3 days later, after no additional treatment, the numbers of tumor cells were 91 to 94% lower than in the control nontreated microcapsules, indicating that the lethal damage to the tumor cells by the drugs was more fully realized several days after treatment. Therefore, in subsequent experiments the drug effect was evaluated 3–4 days after treatment. With additional administration of CTX or ADR the numbers of MOLT-4 cells were further reduced. Treatment with BCNU was comparatively less effective. The numbers of MOLT-4 lymphoma cells continued to increase and even after 2 injections, they were just 10% below control level (Table 5). Ten days after implantation, large numbers of MOLT-4 cells had accumulated inside the microcapsules ($2.7 \times 10^6$ cells/ml of microcapsules). This afforded an opportunity to determine if drug effect in this model could be assessed not only by its ability to inhibit tumor cell proliferation but also to actually reduce the total tumor mass inside the microcapsules. When CTX was applied 10 days after transplantation of microcapsules, a clear cytoreductive effect was observed. The number of tumor cells remaining at day 14 was...
MICROENCAPSULATED TUMOR ASSAY

Table 1  In vivo growth of encapsulated tumor cells after in vitro preincubation with chemotherapeutic drugs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of MOLT-4 lymphoma cells/1 ml of MC</th>
<th>% of inhibition</th>
<th>No. of FEM melanoma cells/1 ml of MC</th>
<th>% of inhibition</th>
</tr>
</thead>
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<tr>
<td>None</td>
<td>6 x 10^6</td>
<td>2.7 x 10^6</td>
<td>96</td>
<td>90</td>
</tr>
<tr>
<td>ADR</td>
<td>0.1 x 10^6</td>
<td>99.8</td>
<td>2.6 x 10^6</td>
<td>72</td>
</tr>
<tr>
<td>BCNU</td>
<td>0.1 x 10^6</td>
<td>98</td>
<td>4.1 x 10^6</td>
<td>88</td>
</tr>
<tr>
<td>5-FUra</td>
<td>1.2 x 10^6</td>
<td>80</td>
<td>4.6 x 10^6</td>
<td>83</td>
</tr>
</tbody>
</table>

* NT, not tested.

Table 2  In vivo inhibition of proliferation of encapsulated MOLT-4 lymphoma cells after i.p. or i.v. administration of chemotherapeutic drugs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days of treatment</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>7 days</td>
<td>3 x 10^6</td>
</tr>
<tr>
<td>CTX</td>
<td>0.2 x 10^6</td>
<td>98</td>
</tr>
<tr>
<td>ADR</td>
<td>0.6 x 10^6</td>
<td>95</td>
</tr>
<tr>
<td>5-FUra</td>
<td>0.8 x 10^6</td>
<td>93</td>
</tr>
</tbody>
</table>

Table 3  In vivo sensitivity of MOLT-4 human lymphoma cells to cytotoxic drugs

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. of tumor cells/1 ml of MC</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>58 ± 2.3</td>
</tr>
<tr>
<td>ADR</td>
<td>2.7 ± 0.2</td>
</tr>
<tr>
<td>CTX</td>
<td>1.9 ± 0.1</td>
</tr>
<tr>
<td>5-FUra</td>
<td>41 ± 1.6</td>
</tr>
</tbody>
</table>

In order to assess whether the antitumor effect of drugs could be evaluated against various histological types of tumor cells, a series of experiments was performed with cell lines derived from breast and lung carcinomas and malignant melanoma. In the first of these experiments ADR (6 mg/kg), VBL (1 mg/kg), 5-FUra (60 mg/kg), and CTX (100 mg/kg) were administered i.v. to mice 1, 5, and 9 days after microcapsule (MC) implantation. The number of tumor cells accumulated inside the MC was determined 4 days after last treatment. Each treatment group consisted of three mice.

12 times lower (2.2 x 10^6) than it was at the time when the treatment started (27 x 10^6 at day 10) (Table 5).

In order to assess whether the antitumor effect of drugs could be evaluated against various histological types of tumor cells, a series of experiments was performed with cell lines derived from breast and lung carcinomas and malignant melanoma. In the first of these experiments ADR (6 mg/kg), VBL (1 mg/kg), 5-FUra (60 mg/kg), and CTX (100 mg/kg) were administered i.v. to mice 1, 5, and 9 days after i.p. transplantation of encapsulated MCF-7 breast adenocarcinoma (Table 6). Progressive growth of MCF-7 cells was observed in the nontreated control microcapsules. Vinblastine had a relatively long lasting antiproliferative effect. While the numbers of tumor cells in the control group increased progressively, those in the VBL group exhibited only slight changes. Correspondingly, the relative differences between VBL-treated and nontreated control groups increased with time of observation: from 22% 2 days after the last treatment to 80% 13 days later. Some drugs had only transitory antitumor effects; for example, 13 days after treatment, the numbers of cells inside ADR- and 5-FUra-treated microcapsules were the same as those in untreated controls. Treatment with CTX yielded a 47% tumor growth inhibition. Encapsulated A549 non-small lung carcinoma cells also showed a high level of proliferation in vivo (data not shown). They were more sensitive to ADR and BCNU (>80% inhibition) than to CTX (50% inhibition).

In examining the ability of various human tumor cell lines to grow inside the microcapsules, we have observed a consistent advantage of in vivo conditions for supporting tumor cell proliferation in comparison to those which were provided in vitro. Table 7 and Fig. 3, A and B, illustrate this phenomenon. Substantially more tumor cells accumulated inside the microcapsules implanted in the peritoneum of mice than in microcapsules cultured in vitro in conventional culture medium for the same time period. Although all of the experiments above used athymic nude mice, further studies indicated that the microcapsules could protect tumor cells sufficiently to permit successful short-term growth in immunocompetent C57BL/6 mice (Table 8). Progressive growth of human MOLT-4 lymphoma cells inside the microcapsules was observed after their implantation into nude or immunocompetent C57BL/6 mice. No difference in tumor cell response to the drugs was found regardless of the immune status of the host (Table 8). In some experiments, microcapsules recovered from C57BL/6 mice later than 8 days after implantation were heavily covered with host peritoneal cells (macrophages, other mononuclear cells with lymphoid morphology, and granulocytes). Treatment of microcapsules with a 0.25% solution of trypsin was effective in removing the adherent host cells. In C57BL/6 mice treated with drugs, the covering of microcapsules with host cells was less obvious and in some cases was not observed at all. Presumably the immunosuppressive action of these drugs abolished the host response to the human antigenic material released through the semipermeable membrane of the microcapsules. In short-term experiments, host cell adherence to the microcapsules did not impair tumor cell proliferation.

The above data indicate that chemotherapeutic drugs can affect the growth and survival of encapsulated tumor cells and that tumor cells demonstrate various levels of sensitivity to drugs. In order to further assess and discriminate tumor cell drug sensitivity by using the META, the in vivo effects of various drugs on the growth of encapsulated murine P388 leukemia cells and a multidrug resistant variant of P388 (P388/ADR) were compared. One day after implantation of microcapsules, mice were treated with drugs (Table 9). Five days after implantation, numerous P388 cells had accumulated in the control, nontreated microcapsules, whereas one treatment with ADR, VBL, or CTX produced dramatic antitumor effects. In contrast, ADR and VBL did not affect growth of the multidrug resistant subline. Cyclophosphamide almost completely prevented the proliferation of both of these cell types (Table 9). After a second treatment with drugs the essential profile of drug sensitivity did not change.

In vivo antitumor activity is usually assessed by the ability of drugs to inhibit the growth of transplanted tumor cells and/or to cure the treated animals. Therefore, the effects of chemotherapeutic drugs on the growth of encapsulated human LOX melanoma cells were compared with their ability to inhibit tumor formation and lethality in nude mice inoculated i.p. with nonencapsulated LOX melanoma cells. Nude mice were treated...
were given injections i.v. of ADR (6 mg/kg) on days 1 and 4. There were two injections i.p. of ADR (10 mg/kg) on days 1 and 4 days after last treatment.

There was a dramatic decrease in the numbers of encapsulated and nonencapsulated LOX melanoma cells (Table 10); drugs 1, 5, and 9 days after i.p. transplantation of encapsulated LOX melanoma cells were either transplanted i.p. into nude mice treated with ADR, BCNU, or CTX. Some mice received CTX 10 days after transplantation and the number of tumor cells was determined 4 days later.

Table 4 Numbers of LOX melanoma cells recovered from individual mice treated or nontreated with Adriamycin

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Days after MC implantation</th>
<th>Mouse no.</th>
<th>Volume of MC (ml)</th>
<th>No. of tumor cells (&gt;10⁶) recovered (% of viable cells)</th>
<th>No. of tumor cells/1 ml of MC (&gt;10⁶)</th>
<th>Mean ± SD (×10⁷)</th>
<th>Coefficient of variation</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>6</td>
<td>1</td>
<td>0.26</td>
<td>4.6 (48)</td>
<td>17.6</td>
<td>17.2 ± 0.8</td>
<td>4.6</td>
</tr>
<tr>
<td>ADR</td>
<td>6</td>
<td>1</td>
<td>0.17</td>
<td>3.3 (46)</td>
<td>16.2</td>
<td>19.7 ± 1.0</td>
<td>5.4</td>
</tr>
<tr>
<td>BCNU</td>
<td>6</td>
<td>1</td>
<td>0.3</td>
<td>4.3 (46)</td>
<td>17.8</td>
<td>19.9 ± 0.6</td>
<td>3.0</td>
</tr>
<tr>
<td>CTX</td>
<td>6</td>
<td>1</td>
<td>0.27</td>
<td>0.4 (73)</td>
<td>1.7</td>
<td>1.8 ± 0.09</td>
<td>5.3</td>
</tr>
<tr>
<td>ADR</td>
<td>6</td>
<td>1</td>
<td>0.29</td>
<td>0.5 (70)</td>
<td>1.8</td>
<td>1.8</td>
<td>5.3</td>
</tr>
<tr>
<td>BCNU</td>
<td>6</td>
<td>1</td>
<td>0.23</td>
<td>0.4 (60)</td>
<td>1.8</td>
<td>1.8</td>
<td>5.3</td>
</tr>
<tr>
<td>CTX</td>
<td>6</td>
<td>1</td>
<td>0.23</td>
<td>0.5 (71)</td>
<td>1.8</td>
<td>1.9 ± 0.1</td>
<td>5.2</td>
</tr>
<tr>
<td>ADR</td>
<td>6</td>
<td>1</td>
<td>0.24</td>
<td>0.5 (77)</td>
<td>2.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5 In vitro response of encapsulated human breast adenocarcinoma cells to drugs

Microcapsule MOLT4 lymphoma cells were encapsulated and implanted into the peritoneum of nude mice. Mice received i.v. ADR (10 mg/kg), BCNU (40 mg/kg), and CTX (200 mg/kg) 6 and 10 days after microcapsule (MC) transplantation. The numbers of tumor cells inside the MC were determined 1 and 4 days after first treatment (7 and 10 days after transplantation) and 4 days after the second treatment. Some mice received CTX 10 days after transplantation and the number of tumor cells was determined 4 days later.

Table 6 In vivo response of encapsulated human breast adenocarcinoma cells (MCF-7) to chemotherapeutic drugs

Human breast adenocarcinoma cells (MCF-7) were encapsulated and implanted i.p. in nude mice. ADR (6 mg/kg), BCNU (1 mg/kg), and 5-FUra (60 mg/kg), and CTX (100 mg/kg) were injected 1, 5, and 9 days after microcapsule (MC) implantation. The numbers of tumor cells in MC were determined 2, 9, and 13 days after last treatment.

Table 7 In vivo and in vitro growth of encapsulated human tumor cells

Tumor cells were encapsulated and were either transplanted i.p. into nude mice, or were cultured in vitro for 12 to 20 days. The numbers of tumor cells inside the microcapsules (MC) were determined on the days indicated.

Table 8 Effect of drugs on the growth of encapsulated MOLT4 human lymphoma cells transplanted into athymic nude or immunocompetent C57BL/6 mice

Human microcapsule MOLT4 lymphoma cells were encapsulated and implanted i.p. into athymic nude or C57BL/6 mice. Mice received i.v. injection of CTX (200 mg/kg) and ADR (10 mg/kg) 2 and 4 days after microcapsule (MC) implantation. Numbers of tumor cells in MC were determined 3 and 6 days after the last treatment.

Table 9 In vivo effect of cytotoxic drugs on the growth of encapsulated P388 leukemia cells

Encapsulated P388 or P388/ADR cells were implanted i.p. into nude mice. ADR (6 mg/kg), VBL (1 mg/kg), or CTX (100 mg/kg) was injected 1 day after implantation of the microcapsules (MC). Five days after implantation (before the second treatment) the MC were recovered and cell counts were performed. An additional determination was performed 3 days after the second treatment (8 days after MC implantation).

with drugs 1, 5, and 9 days after i.p. transplantation of encapsulated and nonencapsulated LOX melanoma cells (Table 10); microcapsules were recovered 3 days after the last treatment. There was a dramatic decrease in the numbers of encapsulated LOX cells in mice treated with ADR, BCNU, or CTX (Table 10). 5-FUra had a less profound antitumor effect with numerous proliferating tumor cells remaining inside the microcapsules. In parallel with these findings, when 5-FUra was administered to mice transplanted i.p. with nonencapsulated LOX melanoma cells, relatively little therapeutic effect was observed (all mice died from the tumor ascites). The other drugs tested had strong inhibitory effects, resulting in survival of 33 to 50% of the mice transplanted with nonencapsulated LOX melanoma cells (Table 10). Comparison of photomicrographs of microcapsules derived from the ADR- and 5-FUra-treated mice clearly demonstrates the differences in sensitivity of LOX melanoma cells to these drugs (Fig. 3, B–D).

As demonstrated above, the META permitted evaluation of the antitumor effect of single versus multiple treatments and assessment of cytostatic or cytoreductive activity of selected drugs. To further explore these phenomena the LOX melanoma
cell line was investigated with single or multiple treatments with ADR or 5-FUra. Treatment was started at different time periods after implantation of microcapsules. Adriamycin (6 mg/kg) administered i.v. 1, 5, and 9 days after implantation of microcapsules containing LOX melanoma cells completely prevented proliferation of tumor cells (Fig. 4A). When ADR was injected 5 days after implantation of the microcapsules, a substantial decrease in the number of tumor cells was found in microcapsules harvested 4 days later. The second application of ADR further reduced the total number of tumor cells although it did not destroy the ability of surviving cells to proliferate. Indeed, 7 days after the second treatment the numbers of tumor cells inside the microcapsules had substantially increased (Fig. 4A). Nine days after implantation almost the entire volume of the microcapsules in the nontreated mice was occupied by tumor cells and their numbers grew slowly during subsequent days. A single treatment of mice with ADR even at the stage of microcapsule saturation, still resulted in shrinkage of the tumor mass, although numerous viable tumor cells remained. This was evidenced by the fact that 5 days after injection of ADR the number of tumor cells started to rise (Fig. 4A). Treatment of mice with 5-FUra 1, 5, and 9 days after microcapsule implantation dramatically inhibited proliferation of LOX melanoma cells but only in a very transitory manner. One week after the last treatment the number of tumor cells in the 5-FUra-treated microcapsules was similar to that in the non-treated microcapsules (Fig. 4B). This could explain why treatment of mice with this drug 1, 5, and 9 days after transplantation of nonencapsulated LOX melanoma cells failed to cure tumor-bearing mice (Table 10). Similarly, when 5-FUra treatment was applied 5 or 9 days after implantation of microcapsules, there was growth retardation, but no reduction in the number of tumor cells below the initial level (Fig. 4B). Thus, in contrast to ADR, 5-FUra caused predominantly a cytostatic rather than a cytoreductive effective on LOX melanoma cells.

**DISCUSSION**

Recent progress in biotechnology made possible the new approach described herein which is based on the utilization of microcapsules with semipermeable membranes (17), somewhat similar in concept with the Millipore diffusion chambers (18–20). The encapsulation of human tumor cells and implantation into the peritoneal cavity of mice provides adequate conditions to support their three-dimensional growth. Some tumor cell lines characterized by a high rate of proliferation, such as MOLT-4 lymphoma or LOX melanoma cells, saturated the interior of the microcapsules by 7 to 9 days after transplantation. For other lines (A549 or MCF-7) a longer period was required. Chemotherapeutic drugs administered i.v. diffused readily into the peritoneum of mice, penetrated the membrane of the microcapsules, and reached the tumor cells and killed them or inhibited their proliferation.

The tumor cells studied varied substantially in their sensitivity to chemotherapeutic drugs. Data obtained using the in vivo META in this respect closely paralleled previously described findings of sensitivity or resistance of tumor cell lines to chemotherapeutic agents in other models. The P388 leukemia cell line selected in vivo for resistance to ADR showed multidrug resistance to other agents such as VBL, using survival as assay end point (16). In the META, wild-type P388 leukemia cells were highly sensitive to CTX, an agent which is not associated with classical multidrug resistance (21).

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**Table 10 In vivo response of human LOX melanoma cells to chemotherapeutic drugs**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose of treatment (mg/kg)</th>
<th>No. of viable tumor cells/1 ml of MC</th>
<th>% of nonencapsulated tumor cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td></td>
<td>100</td>
</tr>
<tr>
<td>ADR</td>
<td>6</td>
<td>160 x 10^6</td>
<td>0</td>
</tr>
<tr>
<td>BCNU</td>
<td>0.7 x 10^6</td>
<td>8</td>
<td>33</td>
</tr>
<tr>
<td>CTX</td>
<td>0.7 x 10^6</td>
<td>90</td>
<td>50</td>
</tr>
<tr>
<td>5-FUra</td>
<td>6 x 10^6</td>
<td>29 x 10^6</td>
<td>60</td>
</tr>
</tbody>
</table>

* MC, microcapsules.
The antitumor effects of drugs on the growth of encapsulated and nonencapsulated LOX melanoma cells were also compared. Adriamycin, BCNU, and CTX had dramatic inhibitory effects on the growth of encapsulated LOX melanoma cells; similarly, these drugs had less inhibitory effect on encapsulated LOX melanoma cells.

The META has several potential advantages in comparison to other available in vivo tests, particularly with respect to its potential applicability to large scale drug screening operations. The assay is relatively simple, inexpensive, and rapid. Most of the microcapsules implanted in mice can easily be recovered, and by providing approximately 600 microcapsules per mouse, each representing an individual tumor mass, a relatively large population sampling can be generated. This accounts, in part, for the high degree of reproducibility between individual mice treated similarly. This permitted evaluation of treatment effect over time using relatively small numbers of animals. For example, treatment effect for a single agent over a 2-week time course could be evaluated by using 6-12 mice (1-2 mice per time point for treated and control groups with sampling on 3 days). This is substantially fewer than usually required for in vivo testing on tumors transplanted s.c. or i.p. (22-24). Our repetitive experiments using META showed high reproducibility of results.

The SRCA (9) has proven useful with selected xenografts for use in large scale antitumor drug testing. Although this assay is relatively short term it still has some serious limitations. The SRCA requires surgical operation for implantation of the tumor graft and can be complicated by certain difficulties associated with evaluation of the results of drug treatment (10, 11, 13). The SRCA is generally used for the study of human tumor lines which must be expanded initially in nude mice to provide a solid tumor mass; the tumor is cut into small pieces suitable for subsequent implantation under the subrenal capsule. This process is logistically complicated and for some tumors can require a very substantial amount of time. A recent enhancement of the SRCA which may alleviate this problem involves the use of fibrin clots for entrapment and transplantation of enzymatically dissociated tumor cells (25).

Although the present data were obtained by mostly using nude mice, the META can also be performed with the use of conventional mice. META can be rapid, and can be used to evaluate the immediate effect of treatment and drug withdrawal. Some drugs had only transitory antitumor effects; when drug treatment was discontinued, tumor cells proliferated extensively, eventually reaching the numbers found in nontreated capsules. Some drugs permanently impaired tumor cell proliferation. Even in the absence of continued treatment with these drugs, tumor cells proliferated very slowly. In these cases, the differences between control nontreated and drug-treated microcapsules have been found even after prolonged periods of observation.

The META also permitted assessment of the effect of single or multiple drug treatments. Shortly (1 to 3 days) after each administration, drug effect on tumor burden could be assessed. None of the current alternative short-term in vivo assays are readily amenable to providing this kind of information.

Since treatments could be applied at different time periods after microcapsule implantation, it was possible to investigate the effect of tumor size on the effectiveness of drug treatment. The assay also permitted discrimination of cytostatic and cytotoxic effects of drugs. For example, ADR had a cytotoxic effect on LOX melanoma cells whereas 5-FUa showed only cytostatic activity for these cells. Future application of this principle may facilitate preclinical studies aimed at identification of schedules and doses of drugs or combinations of drugs which may be useful for eradication of an established tumor mass.

Our results suggest that the microencapsulation technique can be applied to virtually all histological types of tumor cells and provides excellent conditions for their in vivo growth. This assay can be utilized with very slow growing tumors and even with cell lines which fail to form s.c. tumors in nude mice.

The available data indicate that this new short-term assay is useful for measuring in vivo antitumor activity of drugs against a broad range of tumor cell types. This makes it uniquely suited for use in conjunction with a disease-oriented in vitro drug screening program such as that currently under development by the NCI (4, 5).

The most appropriate role for the META in a human tumor cell line based drug screening program may be to serve as an initial in vivo screen to evaluate and prioritize drug leads coming from the in vitro screens. In this application, activity observed in the META could be complemented by further evaluations in other potentially more demanding and discriminating longer-term models, e.g., classical s.c. tumor assays, or new models using orthotopic tumor transplantation (26) or metastatic models (27).

ACKNOWLEDGMENTS

The authors thank John Wine, Paul Chen, Robert Payne, and Miriam Hursey for excellent technical assistance, and Kathy Gill and the NCI-FCRF Central Clerical Pool for typing this manuscript. We would also like to thank the following individuals for providing samples of the cell lines used in these studies: Dr. Kenneth Cowan (MCF-7); Dr. Isaiah J. Fidler (HT-29 and A549); and Dr. Oystein Fodstad (LOX and FEM.).

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