The Microculture-Kinetic (MiCK) Assay: The Role of a Drug-Induced Apoptosis Assay in Drug Development and Clinical Care


Abstract

A drug-induced apoptosis assay, termed the microculture-kinetic (MiCK) assay, has been developed. Blinded clinical trials have shown higher response rates and longer survival in groups of patients with acute myelocytic leukemia and epithelial ovarian cancer who have been treated with drugs that show high apoptosis in the MiCK assay. Unblinded clinical trials in multiple tumor types have shown that the assay will be used frequently by clinicians to determine treatment, and when used, results in higher response rates, longer times to relapse, and longer survivals. Model economic analyses suggest possible cost savings in clinical use based on increased generic drug use and single-agent substitution for combination therapies. Two initial studies with drugs in development are promising. The assay may help reduce costs and speed time to drug approval. Correlative studies with molecular biomarkers are planned. This assay may have a role both in personalized clinical therapy and in more efficient drug development.

Introduction

Biomarkers that can reliably predict response to chemotherapy and other clinical outcomes, such as time to relapse and overall survival, have been sought. Such assays would have uses in both clinical oncology and drug development. Because most chemotherapy drugs induce cytotoxicity via apoptosis, researchers have attempted to develop a robust method that can correlate in vitro drug-induced apoptosis with clinical outcomes. In this work, we summarize our research to develop a chemotherapy-induced apoptosis assay [the microculture-kinetic (MiCK) assay; this assay has also been given the commercial name Correct Chemo].

Assay Methods

For tumor cell preparation, sterile tumor specimens (surgical biopsy, 5 core needle biopsies, effusions, bone marrow, or blood) are sent via overnight delivery to the DiaTech Oncology Laboratory in Montreal, Quebec, Canada. Neoplastic cells are purified from the solid tumors, effusions, or marrow by a series of steps (proprietary process; DiaTech Oncology).

After purification, each tumor cell preparation is analyzed by a pathologist using cytospin preparations and immunocytochemical stains. To be evaluable, each specimen must achieve at least 90% pure tumor cell content and 90% viability by trypan blue exclusion.

Human JURL-MK2 chronic leukemia in blast crisis cell line (DSMZ) is used as a positive control for MiCK assays performed with patient tumor cells. RPMI-1640 medium without phenol red is used, supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. Cell counts and viability are evaluated by trypan blue dye exclusion.

The procedure used for the MiCK assay for apoptosis was adapted from a previously described method (1, 2). After overnight incubation, chemotherapy drugs are added to 96-well or 384-well plates in 2.5 mL (384-well) or 5 mL (96-well) aliquots. The number of drugs or drug combinations and the number of concentrations tested depend on the number of viable malignant cells that are isolated from the tumor specimen. The drug concentrations (determined by molarity) are those indicated by the manufacturer as the desired blood level concentration ± 1 serial dilution if enough cells are available. Following drug addition, the plate is incubated for 30 minutes at 37°C in a 5% carbon dioxide humidified atmosphere incubator. Each well is then overlaid with sterile mineral oil, and the plate is placed into the incubator chamber of a microplate spectrophotometric reader (BioTek Instruments). The apparent optical density (OD) at 600 nm is read and recorded every 5 minutes over a period of 48 hours. Apparent OD increases,
which correlate with apoptosis, are converted to kinetic units (KU) of apoptosis by means of proprietary software (ProApo) according to a previously described formula (3–6).

A typical apoptotic curve generated by the MiCK assay has an initial steep component followed by a plateau and/or decreasing portion of the curve when measuring OD versus time. The initial steep rise in the curve, where apoptosis is measured, is associated with the increased OD. This is primarily associated with the blebbing of the cell membrane, and in part to cytoplasmic and nuclear condensation. The resulting decline and/or plateau of the OD curve indicate various stages of cell disintegration. During apoptosis, intercellular bridges may be apparent on phase-contrast light microscopies, which can also contribute to the apparent OD. The apoptotic curve is illustrated in Fig. 1A.

In the assay, active apoptosis is indicated as >1.0 KU. A drug that produces ≤1 KU is defined as inactive, and the tumor cells are considered to be resistant to that drug (based on previous laboratory correlations of KU with other markers of drug-induced cytotoxicity, such as growth in culture and thymidine uptake).

The MiCK assay results obtained in blinded clinical trials were not sent to the submitting clinical investigators and treating physicians. Results from unblinded clinical trials or specimens used for routine clinical care were sent to the submitting physician within 72 hours of specimen submission. An example of a clinical report is shown in Fig. 1B.

Correlation of the MiCK Assay with Clinical Outcomes

Initial clinical trials in acute leukemia showed the feasibility of the MiCK assay (4, 5). These early trials were the basis for conducting prospective trials of the assay. A blinded, prospective, multicenter clinical trial was conducted in patients with acute myelocytic leukemia (AML). Bone marrow and/or blood samples from patients with AML who were to undergo induction chemotherapy were submitted for the assay. Because this was a blinded trial, the clinicians never knew the results of the in vitro assay. The outcomes were correlated with the results of induction chemotherapy with the standard regimen of anthracyclines for 3 days and cytarabine for 7 days. The results (Strickland S, unpublished data) indicate a good correlation between idarubicin-induced apoptosis in vitro and clinical outcomes of complete response and overall survival. In patients with an intermediate or favorable AML karyotype, there was a trend toward improved relapse-free survival, although this was not statistically significant. Multivariable analyses indicated that the only significant variable that correlated with the improvement in overall survival was the level of idarubicin-induced apoptosis.

The assay was then studied in solid tumors. A blinded, prospective, multi-institutional trial was conducted in ovarian carcinoma. Specimens from patients who had undergone a resection or tumor biopsy were submitted to the laboratory for MiCK assay. Because this was a blinded study, the physicians never knew the results of the in vitro assay. The physicians used whatever adjuvant therapy they believed was indicated (standard therapy, nonstandard chemotherapy, or even no therapy). The patients’ clinical outcomes were correlated with the in vitro response to the chemotherapy that was used (7; unpublished data). The group of patients who received a chemotherapy that showed substantial drug-induced apoptosis in the MiCK assay experienced significantly longer overall survival rates and time to relapse. If the patients received a chemotherapy that showed substantial drug-induced apoptosis in the assay, significantly higher rates of complete or partial response to chemotherapy were observed.

The assay was then tested in a nonblinded clinical trial to determine the pattern of physician usage with the MiCK assay (8). A wide variety of tumor specimens from 44 patients were submitted for MiCK assay. The assay results were sent to the treating physician, who then had the option to use the results of the MiCK assay or to disregard them in developing a treatment
plan and administering the treatment to the patient. The use of the MiCK assay to determine chemotherapy was correlated with clinical outcomes in the patients. The results indicated that 64% of the physicians used the MiCK assay to determine the treatment plan. If the physicians used the assay to determine the chemotherapy treatment plan, a plan for proprietary drug therapy was changed to generic therapy 57% of the time, and generic drugs were changed to proprietary drugs in 11% of cases. In 36% of those patients, the assay was used with other information (e.g., hormonal therapy based on estrogen receptor status or trastuzumab therapy based on Her2 testing). In 50% of the patients, the treatment plan was changed by physicians as a result of the MiCK assay.

Outcomes of treatment were improved when the physicians used the assay results (Table 1). The response rate (complete and partial responses) was higher when the assay was used by the physician (44% compared with a response rate of 6.7% if the assay was not used; \( P < 0.02 \)). Furthermore, the relapse-free interval was significantly longer when the assay was used by the oncologist (8.6 months compared with 4.0 months if the assay was not used; \( P < 0.01 \)). Most importantly, overall survival was significantly longer (10.1 months if the assay was used to develop the treatment plan compared with 4.1 months if the assay was not used; \( P = 0.02 \)).

These results suggest that the MiCK assay may be useful for improving physician decision-making. The clinical settings may include selection of adjuvant chemotherapy [e.g., deciding among breast cancer adjuvant chemotherapy regimens such as cyclophosphamide, methotrexate, and 5-fluorouracil (5-FU; CMF) vs. cyclophosphamide, doxorubicin, and paclitaxel (AC-T) vs. doxetaxel plus cyclophosphamide (TC) vs. cyclophosphamide, epirubicin, and 5-FU (FEC)], selection of therapy for initial tumor recurrence or metastasis (e.g., deciding among colorectal cancer regimens such as FOLFOX vs. FOLFIRI vs. XELOX vs. 5-FU + leucovorin), and selection of therapy for heavily pretreated patients [e.g., deciding among non–small cell cancer regimens such as docetaxel vs. vinorelbine vs. gemcitabine vs. 2 drug combinations vs. phase I studies vs. supportive care only].

### Impact of the MiCK Assay on Drug Development

Two laboratory trials were conducted to determine the potential use of the MiCK drug-induced apoptosis assay in drug development. In one trial, leukemia cell lines were used to test a new drug (drug A) for a pharmaceutical company (unpublished proprietary information). In the HL60 leukemia line, drug A produced significantly more apoptosis (5.9 KU compared with 2.7 KU for idarubicin and 2.8 KU for daunorubicin). In contrast, in the Ramos Burkitt cell line, apoptosis induced by drug A (6.5 KU) was similar to that induced by daunorubicin (5.9 KU) but inferior to that produced by idarubicin (8.5 KU).

In a second study, 2 colon cancer cell lines were used to test pemetrexed activity (9). In both HT29 and LOVO cell lines, pemetrexed and gemcitabine were inactive, but oxaliplatin showed good drug-induced apoptosis. However, the combination of pemetrexed plus gemcitabine produced more apoptosis compared with oxaliplatin, indicating drug synergy.

Although the validation of in vitro results in cell lines by correlating those results with clinical outcomes in patients awaits further study, early results indicate a potential for the assay to be used at several points in drug development. The assay could be used to screen drugs preclinically with different cell lines, to screen human tumor specimens for activity of a new drug alone or combined with standard drugs compared with standard drugs alone, to select tumor types for clinical trials based on in vitro activity of the new drug in human tumor specimens or tumor cell lines, and to select appropriate patients for phase I, II, or III clinical trials. The ability to more carefully focus clinical trials on patients who have a greater chance of responding to a new drug should shorten the time from drug discovery to U.S. Food and Drug Administration approval based on pivotal randomized prospective clinical trials.

### Cost Reduction

The results of the assay were used to model possible chemotherapy cost reductions in 2 settings. The first model was based on different patterns of physician drug use if the MiCK assay was used to select chemotherapy (10). The claims records of chemotherapy expenditures by a large, self-insured employer were used as a baseline control. It was predicted that if physicians had used the best chemotherapeutic drug based on the MiCK assay together with appropriate biotherapy, a 49% reduction in chemotherapy costs (drug plus supportive care plus administration) compared with the cost of the chemotherapy that was actually used would have been achieved.

In a second modeling study (currently in progress), investigators are evaluating the possible drug savings that can be realized when clinicians use the MiCK assay to develop treatment plans incorporating the best chemotherapy (with either generic multisource drugs or proprietary single-source drugs) in recurrent cancers. These model studies justify prospective clinical trials to specifically compare outcomes and drug costs between groups of patients receiving therapy based on the MiCK assay and patients whose therapy has been determined without the use of a MiCK assay (unpublished data, clinical trials in progress at United Healthcare and Walter Reed National Military Medical Center; NCT00286845).

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**Table 1.** Comparison of clinical outcomes according to physician use of the drug-induced apoptosis assay in patients with measurable disease

<table>
<thead>
<tr>
<th>Patients, n</th>
<th>MiCK assay used by oncologist</th>
<th>MiCK assay not used</th>
</tr>
</thead>
<tbody>
<tr>
<td>Response rate (complete response + partial response)</td>
<td>11/25 (44%) ( P &lt; 0.02 )</td>
<td>1/15 (6.7%)</td>
</tr>
<tr>
<td>Relapse-free interval (median)</td>
<td>8.6 months ( P &lt; 0.01 )</td>
<td>4.0 months</td>
</tr>
<tr>
<td>Overall survival (median)</td>
<td>10.1 months ( P = 0.02 )</td>
<td>4.1 months</td>
</tr>
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</table>
Correlation with Molecular Biomarkers

There is overwhelming interest in the development of molecular biomarkers associated with the choice of chemotherapy. Frequently, this involves testing of archival specimens for the presence of molecular abnormalities. Because such molecular changes may evolve over time, the correlation between molecular biomarkers and drug response and clinical outcomes may also change over time. The MiCK assay is currently being compared with molecular biomarker analyses to determine the correlations between in vitro molecular biomarker patterns and patterns of chemotherapy-induced apoptosis.

However, recent observations in renal cell carcinomas indicate substantial tumor cell heterogeneity in molecular genetic changes between the primary tumor site and metastatic sites (11). Because of this mutational intratumor heterogeneity, the phenotypic chemotherapy-induced apoptosis MiCK assay may provide more theranostic information relevant to treatment decisions.

Comparison of the MiCK Assay with Chemotherapy Sensitivity and Resistance Assays

Clinicians and investigators frequently compare the MiCK assay with previously published chemotherapy sensitivity and resistance assays (12–14). However, the MiCK assay is a novel analysis that differs from such previous technologies in substantial ways. Tumor cell purification is more rigorous in the MiCK assay, reducing confounding chemical or membrane changes from nonneoplastic cells. The cellular and intercellular phenomena associated with apoptosis and cell death are more comprehensively measured by the MiCK assay, reducing the possibility of misinterpreting reversible chemical or membrane changes as irreversible cell death. Because the MiCK assay measures phenotypic changes of apoptosis continuously every 5 minutes for 24 to 48 hours, compared with only one analytic time point in chemosensitivity assays, it can more accurately determine irreversible cell death associated with chemotherapy. Despite studies suggesting some correlation between drug sensitivity and clinical outcomes (12), the lack of a consistent correlation between previous chemotherapy sensitivity/resistance assays and clinical outcomes is reflected in clinical reviews in the literature (13) and the conclusion by in the American Society of Clinical Oncology assessment conclusions (14) that prior assays are not sufficiently robust to warrant general use. Because the MiCK assay was not evaluated in prior reviews, and because it is significantly different from chemotherapy sensitivity and resistance assays, it must be evaluated separately from other tests.

Conclusions

The MiCK chemotherapy-induced apoptosis assay is promising. Future studies are warranted and will be focused on prospectively testing different types of tumors as well as larger numbers of patients. Prospective, randomized, multicenter clinical trials are currently in development to compare patients whose therapy has been selected based on the MiCK assay with patients receiving standard therapy. Such prospective, blinded, multicenter studies will probably be focused on patients with breast, colorectal, or non–small cell lung cancer who will receive chemotherapy for first tumor recurrence or for first presentation with metastatic cancer. Patients will randomly receive standard chemotherapy versus the best chemotherapy based on the MiCK assay.

The MiCK assay may have value in reducing the time and costs required for drug development, improving clinical outcomes, and possibly reducing the costs of oncologic care.

Disclosure of Potential Conflicts of Interest

A. Hallquist and M. Perree are employed by DiaTech Oncology. F. Prendergast, R. Herbst, M. Fleisher, J. Rutledge, M. Chernick, and C.A. Presant are paid consultants of DiaTech Oncology. C.A. Presant serves as Director of Medical Oncology for DiaTech Oncology. F. Prendergast, R. Herbst, M. Perree, A. Hallquist, M. Perree, and C.A. Presant have stock options in DiaTech Oncology. The Wshile Oncology Medical Group receives direct research grant funding from DiaTech Oncology (L. Bosserman, S. Rajurkar, and C.A. Presant are employed by Wshile Oncology Medical Group but do not receive direct research grant funding from DiaTech Oncology); E. Salom, S. Strickland, A. Rapits, K. Rogers, D. Davidson, C. Willis, M. Perree, H. Homesley, M. Burrell, and A. Garrett receive research grant funding from DiaTech Oncology.

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Received February 22, 2012; revised April 2, 2012; accepted April 13, 2012; published OnlineFirst August 3, 2012.

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Cancer Res Published OnlineFirst August 3, 2012.

Updated version Access the most recent version of this article at: doi:10.1158/0008-5472.CAN-12-0681

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