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Letter to the Editor

GLUCOSE CONSUMPTION END POINT IN PRIMARY HISTOCULTURE INDICATES RECOVERY OF HUMAN TUMORS FROM DRUG TREATMENT

Dear Editor:

It is well established that individuals with the same histologic type of cancer do not respond uniformly to currently used anticancer agents. Investigators have developed a wide array of in vitro assays utilizing both animal and human cancer models in an effort to understand these biological differences (1,5–7). A further aim of these test systems is to predict the response of individual patients and study the determinants of drug action at the tissue and cellular level to develop improved therapeutic agents and strategies (7).

Hoffman and coworkers have developed a drug-response assay using collagen-sponge-gels to histoculture tumors which allows the maintenance of tissue architecture and function in vitro (14). Histopathological analysis, DNA precursor uptake (labeling index) and ability to reduce tetrazolium dyes have been studied as growth parameters of human tumors in the histoculture assay (2,3,7,8,11,13). However, these end points, although useful, are destructive techniques requiring termination of the culture at the time of analysis. The measurement of glucose consumption, however, is nondestructive, allowing serial determinations over extended periods in culture. In this report, we describe the use of the glucose-consumption end point in the histoculture drug-response assay and the new insights it reveals into the ability of tumors to recover from drug treatment.

We have previously demonstrated a high success rate of growing human urological tumors in histoculture on collagen sponge gel (1,11) and have chosen this tumor type in histoculture to test the end point of glucose consumption. Tumor tissue identified at the time of radical nephrectomy was transported under sterile conditions to the laboratory within 1 hour of excision. Tumor specimens were reduced to 2 × 2 mm pieces with 5 pieces implanted directly onto rehydrated collagen sponge gels (1 × 1 cm^2) (Health Design Indust., Rochester, NY). Each gel occupied one well of a six-well plate (Falcon, Lincoln Park, NJ). 2.5 ml of medium which was 90% Eagle's minimal essential medium (MEM) (Gibco, Grand Island, NY) and 10% fetal bovine serum (Gibco) were added. Gentamycin (Gibco) was present at a final concentration of 50 μg/ml, and cefotaxime (Hoechst, Somerville, NJ) was present at a final concentration of 1 μg/ml. The final volume of medium was sufficient to reach the upper gel surface without immersing it (2,8,13). Covered cultured plates were maintained in a humidified 5%-CO_2 incubator at 37° C. Cultures underwent sterile-media changes every 72 hours.

Fifty μl of culture medium were taken every 24 hours for determination of glucose content in triplicate using the (HK20) assay kit from Sigma (St. Louis, MO). Measurements are made by monitoring the change in optical density at 340 nm due to reduction of NAD through glucose consumption by hexokinase. The glucose content of the medium was plotted as a semilog plot versus time after medium renewal using Sigmaplot (Jandel Scientific, Corte Madera, CA). A simple exponential model of glucose consumption was then fitted to the data with the program Systat (Systat, Inc., Evanston, IL). The half-life of glucose was calculated from the slope parameter of this model using the equation t 1/2 = 0.693/s, where s=slope of the best-fit linear-regression line of the natural log of the glucose concentration plotted versus time. Glucose content of the medium was measured daily for 3 days. The log values over 3 days were plotted vs. time and the slope of best fit line was taken as the glucose consumption rate during the 3-day period (one period) (Fig. 1 *A–C*).

5-fluoro-2′-deoxyuridine (FdUrd) (Sigma) was added in final concentrations of 0.6 μ*M* and 6.0 μ*M*. Continuous exposure to FdUrd for 12 days was chosen to reflect a current clinical protocol for long-term infusion in patients with metastatic renal carcinoma (9). The concentrations of drug chosen represent values that can be achieved clinically (12).

Analysis of glucose consumption demonstrated that FdUrd produced a transient suppression of glucose consumption with recovery to pretreatment baseline by Day 77 post-treatment (Fig. 1 *A–C*). Although growth in this experiment was evident even by gross examination, this growth was slow (over a period of 6 months), and did not appear to change the glucose consumption rate in the controls over the 80 days monitored in these experiments. Individual specimens were fixed in 10% buffered formalin (pH 7.4) for 24 hours and embedded in paraffin. Sections five microns thick were stained with Harris' hematoxylin and eosin. The primary specimen, control and drug-treated specimens were examined by a pathologist (K. T.). Histopathological changes (necrosis, cellular disorganization, degenerative nuclear changes) and intact tumor cells were observed in all drug treated specimens. Such damage was most pronounced in specimens treated at the highest FdUrd dose (6.0 μ*M*), yet even these cultures recovered.

The collagen-sponge-gel-supported histoculture system allowed the study of renal cell carcinoma in vitro for several months, while maintaining tissue architecture present in the original tumor. The histoculture system appears, therefore, to be useful for monitoring the long-term growth of primary renal cell carcinoma and is a suitable system for quantitation of drug effects by determination of glucose consumption rates which is nondestructive and may be used over long time periods. Since the specimen is studied in vitro in the tissue form, use of the system may provide new information on chemotherapeutic effects at the tissue level.

We have made no attempt to determine whether anaerobic or aerobic metabolism of glucose predominates in these tissues. Regardless of the pathway of glucose utilization (4,10), however, glucose consumption rates appear to be quite stable for a given culture,

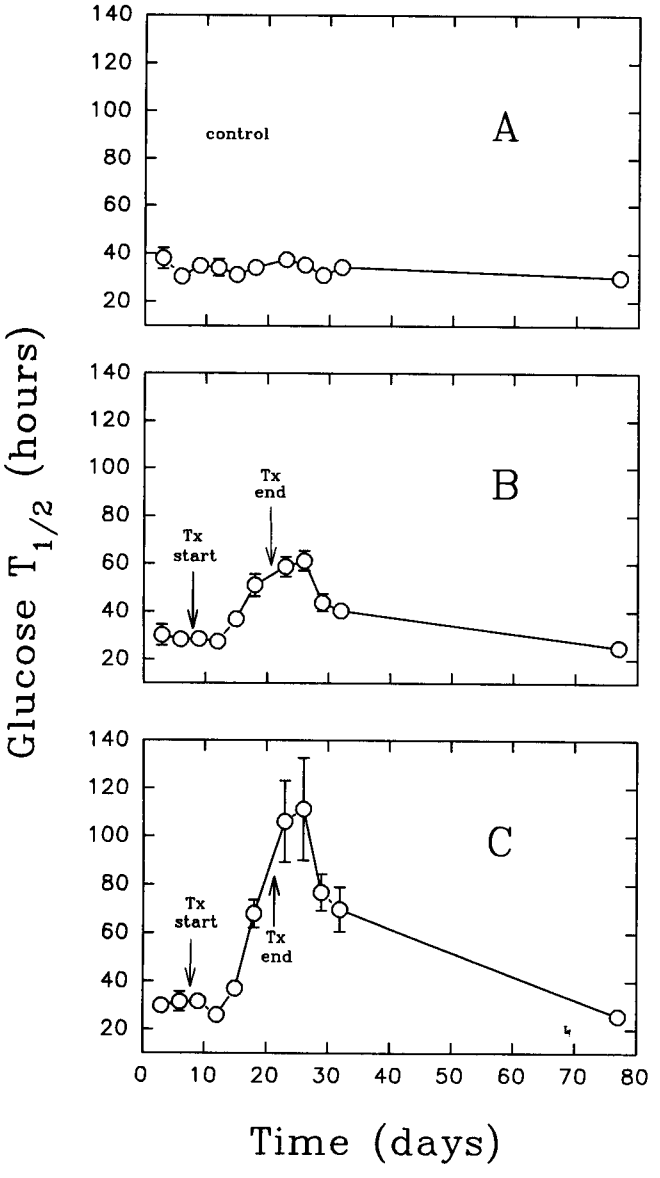

FIG. 1. *A*, Glucose (t 1/2) of control specimen. All specimens were evaluated for two periods (3 days each) prior to initiation of treatment, 4 periods during treatment (in this case media with no drug), and 5 periods post-treatment. Each period represents the medium glucose consumption measured daily for 3 days (one period). *B*, Glucose (t 1/2) of FdUrd-treated specimens. Conditions were similar to those outlined in *A* except treated specimens were exposed to 0.6 μM FdUrd as indicated by the *arrows* in the figure. *C*, Glucose (t 1/2) of FdUrd-treated specimens. Conditions were similar to those outlined in *A*, except treated specimens were exposed to 6.0 μM FdUrd as indicated by the *arrows* in the figure.

enabling each culture to serve as its own control. We view the main use of this measurement to be as a general guide to the metabolic activity of the tissue in vitro, with which it may be possible to apply other measurements. The relationship between glucose consumption rate and other end points traditionally used to determine drug effect in vitro remains to be established. Because the glucose consumption rate can be determined non-invasively, it can be combined with any other end point one might desire.

For example, a study designed to determine drug effect at the time when it is likely to be maximum might require tritiated thymidine probing at or near the nadir of the glucose consumption rate. However, a study designed to determine drug effect in the long-term might require probing after the recovery of glucose metabolism to baseline.

It is notable that in spite of tissue damage due to FdUrd treatment evident in histopathologic analysis, the tissue was able to demonstrate a return to baseline glucose consumption. This implies a cellular heterogeneity in drug effect, with some cells being irreversibly damaged, and others being undamaged or able to recover from damage, so as to restore the glucose consumption rate of the culture. This information appears to be critical in designing treatment strategies that result in actual eradication of tumors.

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