The Measurement of Glucose Consumption in Histoculture to Determine Effects of Doxorubicin and Cisplatinum on Human Gastric Carcinoma

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Abstract. We have developed a chemosensitivity assay of human tumors growing on collagen - sponge - gel - supported histoculture. This assay is thus termed the Histoculture Drug Response Assay (HDRA). In the HDRA, the end points of [³H]thymidine incorporation measured by histological autoradiography and tetrazolium dye reduction were initially used and found to have good in vitro - in vivo correlations, including that determined in clinical trials. We have now developed glucose consumption as an endpoint in histoculture. We have monitored glucose consumption for 11 weeks with histocultured stomach cancer tissue that was obtained from a patient with stomach cancer metastatic to the lymph node. The histocultured lymph node specimens were treated with various concentrations of doxorubicin and cisplatinum. The glucose consumption rate decreased with greater concentrations of both drugs. The results correlated with the thymidine labeling index. From these results, we conclude that the glucose - consumption - rate endpoint in histocultured cancer tissue is non - destructive, unlike the [³H]thymidine and tetrazolium dye end points, allowing serial determinations over extended periods in culture. Thus, the glucose consumption end point may enhance the development of optimal treatment doses and schedules. We also conclude that long - term histoculture drug response studies of metastatic stomach cancer are possible.

It is well established that individuals with the same histologic type of cancer do not respond uniformly to currently - used anticancer agents (1, 2). 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. Collagen - sponge - gel - supported histoculture has been shown to support the growth and native state three - dimensional architecture of both tumor and normal tissue, often for long periods of time (3, 4, 5). This method of histoculture was utilized to develop a chemosensitivity assay, the Histoculture Drug Response Assay (HDRA), for individual cancer patients by assessing the effects of drugs on the patients' histocultured tumor (6 - 9).

Various endpoints to measure drug response have been utilized in histoculture, including [³H]thymidine incorporation measured by histological autoradiography, reduction of tetrazolium dyes, the use of vital dyes to indicate cell viability (10), and glucose consumption to measure metabolic activity (11).

We have previously demonstrated a high success rate of growing human urological tumors and normal kidney tissue in histoculture on collagen - sponge - gels (12, 13). We have described the use of the glucose - consumption endpoint in the HDRA and the new insight it reveals into the ability of tumors to recover from drug treatment (11). In the present study we have applied this system to grow and test the chemosensitivity of the lymph - node metastases of human stomach cancer. The cultures were studied for this response to drugs by visual inspection, incorporation of [³H]thymidine and glucose consumption.

Materials and Methods

Tissue procurement. Lymph node tissue involved with metastatic stomach cancer, identified by frozen section at the time of total gastrectomy, was transported in a sterile container to the laboratory which was near the operating room.

Collagen - sponge - gel - culture (Histoculture). The metastatic lymph nodes were divided into 2 to 3 mm diameter pieces and five pieces were placed on top of previously hydrated Spongostan gels (1 x 1 x 1 cm) (Health Design Indust., Rochester, N. Y.). One gel was put in each well of six well dishes. Three milliliters of Eagle’s minimal essential medium (MEM) (GIBCO, Grand Island N.Y.) supplemented with 10% fetal bovine serum (GIBCO), and 50 µg/ml gentamicin were added to each well. The final volume of medium was sufficient to reach the upper gel surface without immersing it.

Covered culture plates were maintained in a humidified 5% CO₂ incubator at 37° C. The cultures underwent sterile media changes every 72 hours. Histoculture was continued up to 11 weeks after explantation.

DNA precursor uptake. Autoradiography was carried out after the cultures

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Figure 1. Glucose consumption rate on an 11-week histocultured lymph node metastatic stomach cancer specimen. Each period represents the medium glucose concentration at 24 hour intervals over a 3-day measurement period.

were exposed to medium containing 4 μCi/ml [methyl-3H]thymidine for 72 hours. Fixation, embedding, sectioning and deparaffinization were subsequently carried out. Specimen slides were exposed to Kodak NTB liquid emulsion [Kodak, Rochester NY] for 10 days at 4°C, followed by standard development in D19 Kodak developer and staining with Hematoxylin and Eosin.

The DNA labeling index is defined as the number of labelled cells (>4 silver grains/nucleus) divided by the total number of cells (100 cells minimum) evaluated. An effort was made to evaluate only the most active area of all slides to avoid false-positive drug effects due to sampling error.

Drug treatment. Specimens were exposed to media containing 0.1x, 1x, and 10x of achievable human peak plasma concentrations of cisplatinum, doxorubicin and a combination of both drugs for 72 hours. After drug treatment, the specimens were washed with phosphate-buffered saline and fresh media.

Glucose consumption. Fifty μl of culture medium were taken every 24 hours for determination of medium glucose content in triplicate using the HK20 assay kit from Sigma (St. Louis, MO). Measurements were made by monitoring the change in optical density at 340 nm due to the reduction of NAD catalyzed by hexokinase with the glucose substrate before and after chemotherapy treatment. The glucose content of the medium was plotted as a semilog plot versus time after medium renewal using the Sigma plot program (Jandel Scientific, Corte Madera, CA). A simple exponential model of glucose consumption was then fitted to the data with the Systat program (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. The 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 the best-fit line was taken as the glucose consumption rate during the 3-day measurement period (one period).

Results and Discussion

Length of histoculture time. The lymph node metastases of stomach cancer were found to be viable in histoculture for at least 11 weeks as determined by histological autoradiographic measurements of [3H]thymidine incorporation (Figure 5), histological analyses (Figure 7) and glucose consumption (Figure 1). The histoculture system was shown to be useful for long-term studies of responses to drugs and potential recovery from initial responses.

Glucose consumption as endpoint for drug response of stomach cancer. As can be seen in Figure 1, glucose consumption in the control histocultures remained constant over 6 periods of measurement covering 11 weeks of histoculture. This long-term constancy of metabolism of the stomach cancer histoculture enabled the long-term measurement of drug response and recovery with this end point.

Treatment of the stomach cancer histocultures with various concentrations of cisplatinum and doxorubicin elicited the following responses: as can be seen in Figure 2, doxorubicin at 0.06 μg/ml and 0.6 μg/ml had no effect on glucose consumption. However, 6 μg/ml and 60 μg/ml irreversibly arrested glucose consumption. Cisplatinum at 0.24 μg/ml had no effect.
Figure 2. Change of glucose half-life on the 11 week histocultured specimen after treatment with doxorubicin.

Figure 3. Change of glucose half-life on histocultured specimens that were treated with cisplatinum for 72 hours. Note the marked slowing of glucose consumption in cultures treated with 24 μg/ml of cisplatinum.
$[^3]H]$thymidine endpoint to measure drug response in comparison to glucose consumption endpoint in histoculture. As can be seen in Figure 5, the effects of doxorubicin and cisplatinum tested alone had parallel effects on glucose consumption and $[^3]H]$thymidine uptake on histocultured stomach cancer, (see also Figures 8,9,10). However, treatment with the combination of doxorubicin at 0.06 μg/ml and cisplatinum at 0.24 μg/ml (Fig. 11) as well as the combination of doxorubicin 0.6 μg/ml and cisplatinum at 2.4 μg/ml had no effect on $[^3]H]$thymidine incorporation, even though these combinations had effects on glucose consumption. The high concentration of 2.49 μg/ml cisplatinum eliminated $[^3]H]$thymidine incorporation (Figure 5) and it also completely arrested glucose consumption. Thus glucose consumption may be sensitive as an end point than

Figure 4. Histocultured specimens were treated with doxorubicin combined with cisplatinum. The glucose half - life was increased. However the glucose consumption rate recovered by posttreatment period 6 after the minimum - treatment doses were applied.


This potential increase in sensitivity of the glucose end point, combined with fact that it is non - invasive, allowing
Figure 6. Gross histologic findings after 11 weeks of histoculture of the lymph node metastasis of human stomach cancer.

Figure 7. Histologic findings of the control group of after 11 weeks of histoculture of the lymph node metastasis of stomach cancer. The histocultured specimen exhibits morphological characteristics of cancer cells and high uptake of thymidine. The collagen gel has been infiltrated by cancer tissues (autoradiography and H-E x 200).
Figure 8. The histocultured specimens were treated with 0.06 μg/ml of doxorubicin. Autoradiograms reveal high incorporation of [3H] thymidine.

Figure 9. The specimen was treated with 60 μg/ml of doxorubicin. Autoradiogram reveals only pyknotic cells without cell survival.
Figure 10. Autoradiogram of histocultured specimens treated with 0.24 μg/mL of cisplatinum reveals good cell survival and thymidine incorporation.

Figure 11. The histocultured specimens were treated with 0.05 μg/mL of doxorubicin combined with 0.24 μg/mL of cisplatinum. The autoradiogram reveals cell viability.
multiple measurements on the same histocultures, gives rise to the possibility of determining in vivo clinically important information for individual cancer patients.

The following conclusions can be made from these studies: a) long term histoculture drug response studies of metastatic stomach cancer are possible; b) the glucose consumption rate end point in histocultured lymph node metastatic stomach cancer tissue is non-destructive, unlike the [3H]thymidine and tetrazolium dye endpoints, allowing serial determination for extended periods in culture after treatment; c) the glucose - consumption endpoint may be more sensitive than the [3H]thymidine incorporation end point, with the glucose - consumption end point revealing subtle differences between treatment regimens and d) the glucose - consumption endpoint may enhance the development of optimal treatment doses and schedules of chemotherapy.

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References


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