Dissemination of Melanoma Cells within Electrocautery Plume

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BACKGROUND: The observed occurrence of port site recurrence in laparoscopic surgery for malignant disease has stimulated interest in the dissemination of tumor cells during surgery. Study of electrocautery smoke has revealed the presence of large particles and viable viruses. The purpose of this study was to determine if viable malignant cells are present in suspension within the electrocautery plume.

METHODS: Pellets of B16-F0 mouse melanoma cells were cauterized and the plume collected into culture medium. In part 1 of this study, the trypan blue assay was used to assess cell viability immediately after collection and 7 days later. A cautery current of 30 W was applied for 5 minutes. In part 2, the tetrazolium (MTT) viability assay was used to assess cell viability after cauterization of tumor pellets at 10, 20, and 30 W for 5 seconds.

RESULTS: Although intact melanoma cells were identified with the trypan blue assay immediately after plume collection, no viable cells were seen at 7 days using this assay. In part 2, viable melanoma cells were present in the culture wells at 7 days. Lower fulguration currents appeared to yield higher cell counts: 2,250 cells/well at 10 W, 2,100 cells/well at 20 W, and 1,800 cells/well at 30 W.

CONCLUSIONS: Results of this study confirm that application of electrocautery to a pellet of melanoma cells releases these cells into the plume. These cells are viable and may be grown in culture. This release of malignant cells may explain the appearance of port metastases at sites that are remote from the surgical dissection or that were never in direct contact with the tumor.
***Am J Surg.* 1999;178:57–59.**

Following the introduction of laparoscopic cholecystectomy, it was hoped that this surgical approach could be applied to a number of intra-abdominal surgical interventions. Among others, the technique of colonic resection was developed and became popular with several surgeons. However, port site recurrences of the primary cancer loomed as a dark cloud over these generally encouraging results, leading to recommendation by some authors that laparoscopic colonic resections for malignant conditions be performed within the confines of controlled clinical trials only. The incidence of port site recurrence has been reported as 0.69% in open procedures and as high as 1.4% in laparoscopic cases.[1] The incidence in laparoscopic surgery is lower (0.48%) in the absence of diffuse peritoneal metastases.[2] Although the lower incidence of metastatic incisional recurrence in open surgeries may be due to a failure to report cases, the circumstances of reported cases led us to suspect that there are indeed physiological conditions specific to laparoscopy that promote port site recurrence.

The mechanism of port site metastases has not been elucidated, and numerous hypotheses have been proposed, including intraoperative manipulation of tumor, instrument contamination, and cellular aerosolization within the pneumoperitoneum. Also, there is experimental evidence to suggest that the CO_2, frequently used to create pneumoperitoneum enhances growth of tumor cells and facilitates their implantation into the abdominal wall after resection of malignant tumors.[3]

Another factor may be that cauterization of a tumor releases malignant cells within the electrocautery plume. These suspended cells would then be able to disseminate within the peritoneal cavity and implant at distant sites. Two observations lend support to this hypothesis. First, microscopic examination of the cautery plume reveals the presence of cellular and organic material in the range of 2 to 25 μm, which is well within the range of living cells.[4] Second, the viability of some of this cellular debris has been shown by the identification and growth of viruses collected from the plume.[5,6] Biologically active cells within the smoke have not yet been identified.[7]

The purpose of this study was to determine whether application of electrocautery to malignant tumors would result in liberation of viable malignant cells within the smoke. The study was conducted in two parts. The objective of the first part was to determine qualitatively if live malignant cells were present in electrocautery plume. The objective of the second part was to quantify the number of live malignant cells and evaluate the effect of varying fulguration current on the number of viable cells.

MATERIALS AND METHODS

Cell Line

B16-F0 mouse melanoma cells ATCC No. 6322 are cultured in Dulbecco's modified Eagle's medium with 4.5 g/L

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Manuscript submitted December 17, 1998, and accepted in revised form April 19, 1999.

glucose, 90%, and fetal bovine serum 10% and penicillin/streptomycin at 37°C in a 5% CO_2 incubator.

Part 1: Identification of Live Melanoma Cells in Cautery Plume

Evaluation of viable tumor cells from the cautery plume was performed both in vitro and in vivo. In the in vitro experiment, centrifuged tumor pellets containing 3.8×10^7 B16 melanoma cells were cauterized at 30 W for 5 minutes in a sealed environment. All procedures using electrocautery were performed with a Birtcher 4400 Power Plus electrosurgical generator. The collection chamber containing the sterile culture media was connected to a suction apparatus/generator under aseptic enclosed conditions. The suction apparatus generated a suction force of 10 to 15 mm Hg. The smoke was collected through 7/16" OD Tygon(r) sterile tubing (30 cm in length) and bubbled through 200 cc of sterile cell culture media. The smoke was analyzed for the presence of viable cells by the trypan blue assay and tumor growth by visual inspection for tumor colonies over a period of 6 to 8 weeks.

Part 2: Quantification of Melanoma Cells in Cautery Plume

In part 1 of the study, intact melanoma cells were identified in the immediate postcautery period. However, by the trypan blue assay, most of these appeared to be nonviable. The goal in part 2 was to objectively quantify the number of viable melanoma cells in the smoke aerosol. To do so, the tetrazolium (MTT) viability assay was used. This assay was selected because of its high degree of precision.[8] Since tetrazolium is cleaved in the mitochondria of living cells, the demonstration of this activity is an indication of the presence of living cells. Dead cells do not actively cleave MTT owing to their inactive mitochondria. Cleavage of MTT by living cells produces a colored byproduct, formazan, which can be objectively quantified in the enzyme-linked immunosorbent assay (ELISA) plate reader at 560 nm. The quantity of this byproduct is a reflection of the number of living cells present.

To standardize the viability assay, a control pellet of noncauterized tumor cells containing 1×10^6 cells/mL was prepared and serially diluted for the tetrazolium (MTT) viability assay. The serial dilutions were plated in 4 separate quadrants in 96-well flat-bottom culture plates and read after 5 to 7 days of growth in culture. This provided colorimetric standard values for measuring different concentrations of viable cells.

Three pellets (A, B, and C) containing 2.3×10^7 melanoma cells/mL were prepared. Pellets A, B, and C were cauterized at fulguration currents of 10, 20, and 30 W, respectively. Each pellet was cauterized for 5 seconds in total, and the plume was bubbled through 2 cc of culture media in the collection flask. This solution was then plated in the 96-well flat-bottom culture plates. Each well contained 200 μL plume solution and an additional 200 μL media to prevent drying out. The cauterized pellets were scraped off the grounding plates and resuspended in 1 cc culture media and then serially diluted for MTT viability assay to assess viability of the cauterized pellet. The MTT viability assay of the cauterized pellets and their respective plume aerosols were analyzed at 5 days.

The mean spectrophotometric value at each cautery current was the mean result from 10 different sample wells for the cultured cautery plume and serial dilutions of the cauterized pellets. The mean number of viable melanoma cells was determined by linear correlation to the control MTT serial dilution assay.

A negative control was obtained by applying cautery to plain white paper and bubbling the plume through the culture media. This preparation was collected and incubated under the same experimental conditions described above.

RESULTS

The smoke aerosols collected in part 1 were analyzed for the presence of viable cells using the trypan blue viability assay. Large quantities of cellular debris were found in the smoke aerosols, consisting mostly of amorphous charred material and a few morphologically intact cells. Viable cells (intact cells that did not stain with trypan blue) were demonstrated in the initial postcauterization period. However, subsequent culture of the cautery plume did not reveal any growth at 7 days postcauterization.

In part 2, pellets A, B and C contained 2.3×107 melanoma cells per 1 mL volume prior to being cauterized. Approximately 2,250 cells/well were measured from the 10 W cautery plume cultures. At the fulguration current of 20 W, the mean cell count in the cautery plume cultures was 2,100 cells/well. At the fulguration of 30 W, the mean cell number was 1,800 cells/well. Measurement from the cultured pellets revealed 5,000 cells/well for the one cauterized at 10 W, 4,900 cells/well at 20 W, and 4,500 cells/well at 30 W. Although these differences seem to indicate that lower current yield greater cell counts, this was not statistically significant. No viable cells were present in the negative control smoke collection assay.

COMMENTS

Intact viable melanoma cells were identified in the culture media immediately following smoke collection in part 1 of this study. Presumably owing to high fulguration currents and lengthy cautery times, the vast majority of aerosolized particles in the postcauterization period were not viable according to the trypan blue assay. The cautery currents were varied in the second phase of the experiment in order to determine their individual effects on cell viability. Although 5 minutes represents the upper limit of cautery time during surgery, persistent application at one site may raise local tissue temperature excessively and damage large quantities of cells.[4] In the second phase of the experiment, cautery application time was decreased to 5 seconds in order to simulate the short controlled bursts used in surgery and decrease thermal injury to viable melanoma cells.

In previous studies using adenocarcinoma cells, no viable tumor cells were found within the smoke.[7] The trypan blue assay, used in these studies and in part 1 of this study, has been criticized in the literature as being overly sensitive in estimating cell death since the test depends on dye passing through the cell wall due to membrane damage. If cell membrane damage is minimal, the cell may undergo repair and maintain its viability. As a result, in vitro cell growth may be difficult to assess in the early postcauterization period. Another criticism of the trypan blue viability assay

is the inability to accurately quantify the number of viable cells in the plume due to the large volumes of culture media (200 mL) used to collect the plume aerosol. The MTT assay is more sensitive for viable cells, as the conversion of the tetrazolium salt to the deep blue colored formazan requires intact intracellular mitochondria. Dead cells are unable to produce this color reaction, as indicated by previous studies.[8]

In part 2 of this study, viable cells in the plume aerosols were identified in quantifiable numbers 1 week after cauterization. The results indicate a trend in which lower cautery levels (10 W) yield higher mean concentrations of viable melanoma cells within the plume. Higher cautery levels (30 W) may cause increased thermal injury to viable melanoma cells and lead to decreased viable cell concentrations in the plume.

Direct implantation of tumor cells by contact at the port site has also been suggested as a mechanism of abdominal wall metastases. Port site recurrences, however, have been found at ports not directly in contact with cancerous tissue or even when specimen extraction bags have been used. The dissemination of living cells within the peritoneal cavity has been shown to be facilitated by CO_2 pneumoperitoneum.[3] This supports the notion of a "chimney" effect in which aerosolized malignant cells are carried to the port sites as the gas is evacuated, causing port site metastases.[9,10] In this in vitro study, we have demonstrated viable melanoma cells within the electrocautery plume, suggesting that malignant cells may be aerosolized by application of cautery to tumor-bearing tissues.

Results of this study demonstrate that aerosolization of malignant cells occurs as a result of application of electrocautery to malignant tissues. This finding lends support to the hypothesis that the higher incidence of port site recurrence in laparoscopic surgery for malignant disease may be due to implantation of cells in suspension within the electrocautery plume.

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