The effect of lavage on intraabdominal cell burden

S. M. Brundell, K. Tucker, B. Chatterton, P. J. Hewett

1 Department of Surgery, The University of Adelaide, The Queen Elizabeth Hospital, Woodville South, South Australia, Australia
2 Department of Nuclear Medicine, The Royal Adelaide Hospital, North Terrace, Adelaide, 5000 South Australia, Australia

Received: 7 September 2001/Accepted in final form: 8 November 2001/Online publication: 9 April 2002

Abstract
Background: Abdominal lavage is a common surgical practice, but few studies have been conducted to assess its efficacy at removing cells from the abdominal cavity, particularly during laparoscopic surgery.

Methods: After three 12-mm trocars were inserted into six female 30-kg pigs at the umbilicus left and right iliac fossae, the abdomen was insufflated with carbon dioxide. The pelvis of each pigs was injected with 6 million radiolabeled LIM 1215 cells. Then the abdominal cavity was irrigated with either 500 ml 0.9% saline, 500 ml 10% betadine solution, or 1 L 0.9% saline. A maximum of 5 L of solution was used for each animal. The lavage fluid was suctioned into separate containers after each aliquot, and each container was measured for radioactivity.

Results: Significantly greater numbers of cells were removed by lavage by the first to third lavage cycle; however, after four lavage cycles, relatively few cells were removed by each further cycle. No difference was observed between 500-ml and 1-L aliquots. Additionally, the mechanical efficacy of 0.9% saline and 10% betadine solution appeared similar.

Conclusion: These findings suggest that optimal lavage consists of four irrigation/suction cycles utilizing 500-ml aliquots.

Key words: Lavage — Peritoneum — Laparoscopy — Laparotomy — Tumor cell — Bacteria — Abdominal

Since the first description by Price in 1905 [10], intraoperative peritoneal lavage has been widely performed the removal of both bacteria and tumor cells from the abdominal cavity. Many different alternative lavage solutions have been evaluated for additional bactericidal [11] or tumorcidal properties [8] in addition to the mechanical washing effect obtained by repeated lavage.

However, there is still no consensus as to the optimal solution for lavage or indeed for the number of lavage cycles required to remove intraabdominal cells. Therefore, we investigated the mechanical effect of intraoperative lavage and its efficacy in removing intraabdominal tumor cells.

Methods

Experiments complied with the code of practice for the care and use of animals in research in Australia, as formulated by the National Health and Medical Research Council in 1997. The protocol was approved by the Animal Ethics Committee of the Institute of Medical and Veterinary Science, the University of Adelaide, and the Queen Elizabeth Hospital.

Cell labeling

LIM 1215 human colon cancer cells were grown in culture as previously described until they were near confluent across the base of the culture flasks [5]. Free cells were suspended in 20 ml phosphate buffered saline (PBS). An aliquot of this solution was taken and examined in a hemocytometer to calculate cell numbers. A further small aliquot was incubated for 3 min with 0.02% trypsin blue solution (Flow Laboratoraries, Sydney, NSW, Australia) to calculate cell viability. The cell inoculum was then adjusted to provide 6 million cells in 10 ml PBS by diluting the total suspension. The cells were radiolabeled by the addition of 1 GBq off<sup>99m</sup>Tc exametazime (Ceretec; Amersham, Castle Hill, NSW, Australia). The radiochemical purity of exametazime labeling was checked according to a minicolumn method.

Surgical procedure

Female 30-kg domestic pigs were premedicated with intramuscular ketamine (300 mg) (Troy Laboratorities, Sydney, NSW, Australia). Anesthesia was maintained by halothane (induction, 3% maintenance, 1.5%) (Zeneca, Macclesfield, England) via a cuffed endotracheal tube. The pigs were secured to an operating table in a supine position with Millipore tape.

A 12-mm umbilical trocar (Ethicon, Johnson & Johnson Medical, North Ryde, Sydney, NSW, Australia) was inserted using an open
Fig. 1. Percentage of cells remaining in the peritoneal cavity after each cycle of lavage. Values charted are means; error bars mark values for each animal.

technique, and the abdomen was insufflated with carbon dioxide to a pressure of 10 mmHg. A 10-mm 0° telescope was passed into the abdominal cavity and additional 12-mm trocars were inserted in the left and right iliac fossae under direct vision. Using a 19-G needle, million radio-labeled LIM 1215 tumor cells in 10 ml PBS were then injected through the abdominal wall into the pelvis of each pig.

The pigs were then divided into three groups (total, six pigs). Group 1 (two pigs) received 500-ml aliquots of 10% betadine (Apex Laboratories, Somersby, NSW, Australia), group 2 (two pigs) received 500-ml aliquots of 0.9% saline (Baxter Healthcare, Toongabbie, NSW, Australia), and group 3 (two pigs) received 1-L aliquots of 0.9% saline (Baxter Healthcare). A total of 5 L of irrigation fluid was used in all groups, comprising 10 cycles of 500 ml in the pigs in groups 1 and 2 and only five cycles of 1 L in the pigs in group 3.

A 5-mm irrigation/suction device (Ethicon, North Ryde, NSW, Australia) was then passed through the left iliac fossa port. The relevant aliquot of fluid was used to irrigate the abdominal cavity, with particular attention paid to the pelvis. Pigs were kept in a constant position. Suction was then used to extract this aliquot of fluid from the abdominal cavity into separate disposable 3-L suction bags for each aliquot of lavage fluid. Suction was continued until >90% of the aliquot had been removed. The pigs were then killed by intravenous injection of 6500 mg phenobarbitone (Virbac Australia, Sydney, NSW, Australia).

The individual aliquots of lavage fluid were placed on a gamma camera using a low-energy general-purpose collimator (LEM; Serle Nuclearics, Chicago, IL, USA) connected to a computer (Microdelta; Siemens, Hoffmans Estates, IL, USA). Quantitative images were acquired by the computer over a 2-min period. Additional images had previously been created of the background gamma radiation in the laboratory prior to commencement of the study and of the activity of each syringe of radiolabeled cells.

The data were first converted to an interleaved 2b format. Then the resulting images were analyzed with a proprietary written program on a Macintosh PC. The detected counts within a user-defined area were determined, and by subtracting the observed background activity and correcting for radioactivity decay, the true count was calculated. The number of cells present in each aliquot was obtained by multiplying the derived number of counts in the aliquot by the numbers of cells present per count in the original syringe.

Cell resolution was calculated assuming a poisson distribution of radioactivity; therefore, a significant change in radioactivity was calculated as being greater or equal to background activity + (3 × √background activity).

A further assumption was made that this change would be observable on image analysis, giving the cell resolution for each study.

Statistical analysis

Data were analyzed on an IBM-compatible PC utilizing StatsDirect 1.612 (Ashwell, Hertfordshire, England). Data comparing the number of cells retrieved in each lavage cycle were analyzed using a Mann-Whitney U test; variance between animals and groups were analyzed using a Tukey analysis of variance (ANOVA) test. Probability values of <0.10 were taken to be significant.

Results

Cell viability was >95% and Cererec labeling efficiency was >85% for all experiments. Average cell resolution was 45 cells (range, 12–200) for these studies.

The most efficacious lavage was obtained in a pig receiving repeated lavage with 500-ml aliquots of saline; however, even in this animal, only 63% of the original tumor inoculum was obtained (Fig. 1). In most animals, the residual tumor cells were visualized in the pelvis after the completion of all lavage cycles (Figs. 2 and 3): After four lavage cycles, 50% of the tumor inoculum was retrieved from the abdominal cavity (fig. 1). Significantly different numbers of tumor cells were removed by successive lavage cycles until the fourth cycle was compared with the fifth (Table 1). No significant differences were observed between animals or by increasing the aliquot size from 500 ml to 1 L (ANOVA, p = 0.84 to p = 1) (Fig. 4).

Table 1. Each box indicates the calculated probability that differences between the row and column number of abdominal lavage cycles were due to chance

<table>
<thead>
<tr>
<th>Number of Lavage Cycles</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td>0.004</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.001</td>
<td>0.07</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.001</td>
<td>0.07</td>
<td>0.07</td>
<td>0.08</td>
<td>0.05</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.001</td>
<td>0.03</td>
<td>0.07</td>
<td>0.21</td>
<td>0.08</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.001</td>
<td>0.03</td>
<td>0.08</td>
<td>0.21</td>
<td>0.12</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.004</td>
<td>0.03</td>
<td>0.05</td>
<td>0.08</td>
<td>0.12</td>
<td></td>
</tr>
</tbody>
</table>

Fig. 2. Initial picture of radiolabelled cells filling pelvis of pig before lavage.